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THE AMERICAN REVIEW OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF THE NATIONAL TUBERCULOSIS ASSOCIATION

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(Formerly THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES)

ANNUAL MEETING, LOS ANGELES, CALIFORNIA, May 15–20, 1960

See page 2 of advertisements

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Medical Section of National Tuberculosis Association

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Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in tuberculosis and other respiratory diseases may apply for membership in the American Trudeau Society. The dues for active membership in the Society are $18 per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

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Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

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Exhibits will be in the Statler Hilton and will open for viewing at 8:30 AM, MONDAY, MAY 16.

Special interest sessions will begin SUNDAY, MAY 15. *Medical, public health, and nursing sessions* will begin MONDAY, MAY 16.

General sessions will include an opening keynote program Monday at 9:00 AM, followed immediately by the awarding of the Trudeau and Will Ross medals; a session on Monday afternoon at which results of the Arden House Conference on Tuberculosis will be presented (the opening portion will be of special interest to doctors); a session Tuesday morning, and the NTA Business Luncheon on Thursday.

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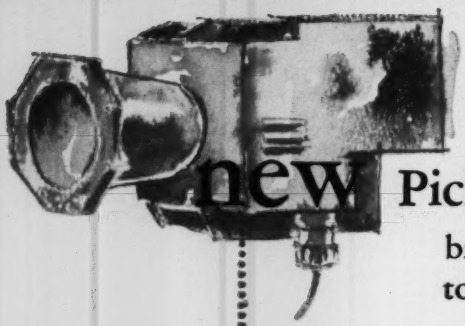

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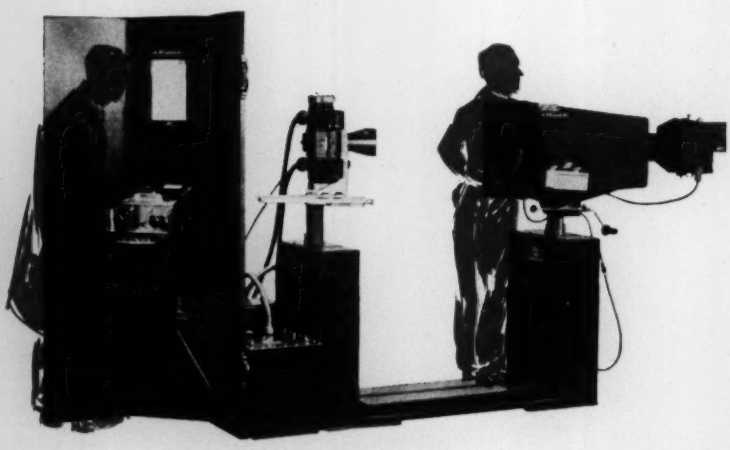

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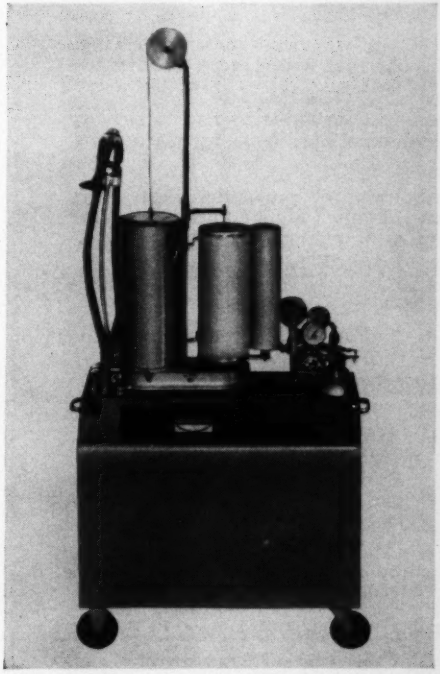

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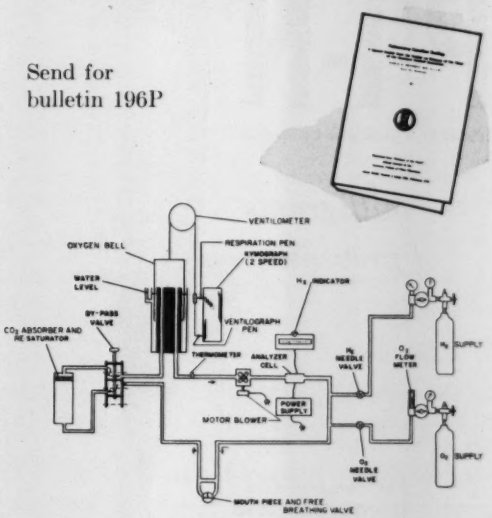

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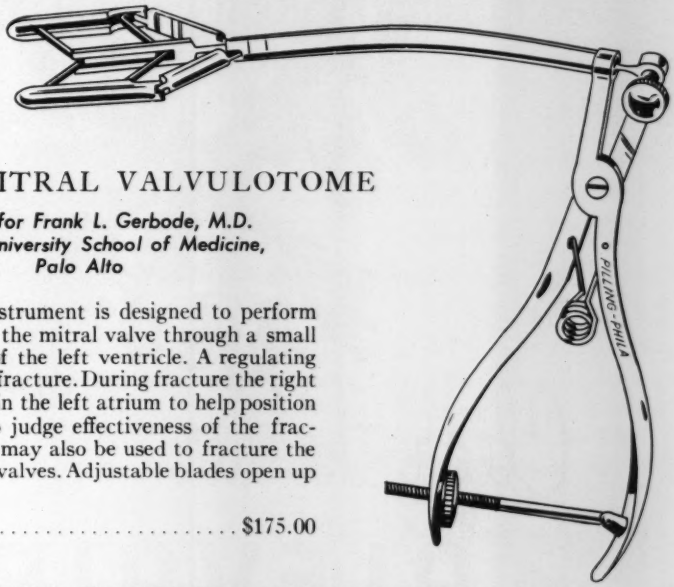

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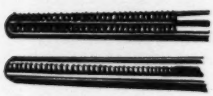

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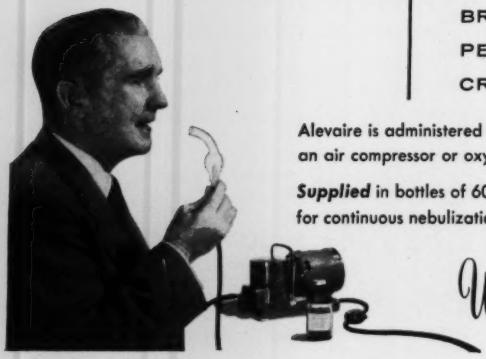

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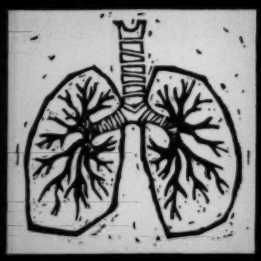

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THE AMERICAN REVIEW
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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 81 *April 1960* NUMBER 4

FOREWORD TO ARDEN HOUSE CONFERENCE RECOMMENDATIONS

Within the last year, a decade and a half after the discovery of the first antituberculosis drugs, two national groups directly concerned in this country with the control of tuberculosis recognized that it is not out of the question to get rid of the disease entirely. In March, 1959, at a meeting of the Tuberculosis Control Advisory Committee of the Public Health Service, in which representatives of the Service and of the office of the National Tuberculosis Association participated, it became apparent that leaders in each group had reached the conclusion independently that now is a ripe time for an all-out program leading to this revolutionary end.

No claim was laid to originality in the concept of final elimination of tuberculosis. Extrapolation of descending mortality curve to a zero end point has intrigued tuberculosis workers for years. But this exercise has been more in the spirit of admiration of the gratifying course that nature is taking than as a lead for long-range planning. And, indeed, to avoid raising hopes that later may be dashed, responsible tuberculosis campaigners have adhered strictly to the word *control* and have studiously avoided any talk of *eradication*.

Soon after the meeting in March, each group, fortified by the knowledge that it would not be charged with heresy by the other in entertaining a concept once considered visionary, made bold to offer specific proposals and recommendations for the conduct of a comprehensive and rapid wind-up campaign. It was obvious at once that each planned to depend in certain respects on the other, and so the most natural first step was to join forces. All agreed that a high-level planning conference of experts was essential, not simply because it is traditional to call a conference or appoint committees when a major new move is contemplated, but for the very good reason that neither group felt omniscient for the task. The specific hope was voiced that men and women of proved ability in other lines of welfare and academic competence, if offered the chance, would come forth with fresh ideas to supplement the patterns of professional tuberculosis control that have become stereotyped in day to day usage.

The result was the Arden House Conference on Tuberculosis, in which an invited group, representing a wide diversity in specialization but a common capacity for original thinking, deliberated for three days of closed sessions in an environment free from outside distraction. To select them in the first place and save time in getting down to work, a small group of staff from each organization met on schedule from early last summer to late fall putting together what was certain to be needed as background for discussion, keeping out what would probably be extraneous, and setting up what were hoped to be practical agenda for the meeting.

The conference took place as planned. The conferees were enthusiastic in their contributions. Argument was spirited and discussions were protracted. Remarkably, after three days of give and take, a unanimity in proposal for action evolved. The official recommendations of the Conference are presented in the following pages, developed through the good offices of a recorder who would have preferred to spend his full time in personal participation in the argument.* It includes a set of unqualified recommendations by the conference, which if once followed will admit of no turning back. The proposal is reminiscent of the eradication campaign in cattle, with germ-destroying chemotherapy to restrict the spread of infection in place of destruction of the individual. But that is getting ahead of the story, which should be studied without haste and with a sober sense of responsibility by all concerned with the conquest of tuberculosis. The proposals are clear.

ESMOND R. LONG

* A condensed summary of the discussions is available in the February issue of the BULLETIN of the National Tuberculosis Association.

RECOMMENDATIONS OF THE ARDEN HOUSE CONFERENCE ON TUBERCULOSIS

The major recommendation of the Conference is a program for the widespread application of chemotherapy as a public health measure for the elimination of tuberculosis in the United States:

Goal: To sterilize that important part of the reservoir of tubercle bacilli that presently exists throughout the country in persons currently suffering from active tuberculous disease, whether presently known or unknown to public health authorities, and in selected persons who previously have had active disease and were inadequately treated.

Technique: Mobilize all resources for a widespread application of the scientifically demonstrated and medically accepted procedures of adequate chemotherapy. These include the proper dosage of appropriate drugs or combination of drugs given continuously over an adequate period of time—procedures that are known to destroy tubercle bacilli in the human body, render the patient's disease noncommunicable to others, and minimize the possibility of reactivation.

The unique aspect of this recommendation lies in its emphasis on such chemotherapy *primarily as a public health tuberculosis control measure* (as well as for the benefit of the individual patient) with all of the connotations of community mobilization and control by public health authorities that this new concept implies. This recommendation obviously implies as well an adequate case detection program.

In addition, the Conference considered some of the deficiencies of current tuberculosis control programs. With regard to strengthening these programs, it made the following recommendations without designating priorities:

1. Assumption by state and local public health authorities of their responsibility for ensuring adequate treatment and rehabilitation of all patients with tuberculosis. Regardless of the place of treatment of the patient, the attending physician should have available to him expert consultation in the fields of both the medical sciences and the social services, which should be integrated in such a manner as to provide continuity of care until the patient is restored to maximum possible productivity.
2. Concentration of the tuberculosis control program on those segments of the population with the greatest tuberculosis problem. This would include re-examination of tuberculosis control grants to states by the Federal government in recognition of the fact that there are strongholds of tuberculosis in the country that are in need of extra resources.
3. Establishment of intermediate goals, en route to the elimination of tuberculosis, together with corresponding suggested program priorities and performance standards. Such national goals and corresponding performance standards should be established as soon as possible by the joint staffs of the National Tuberculosis Association and the United States Public Health Service, following whatever consultation with other individuals and groups seems indicated.

   At the same time the Conference recommended that each state health department, during 1960, formulate its own intermediate tuberculosis control goals, with corresponding program priorities and performance standards, in accordance with a suggested outline to be prepared by the U. S. Public Health Service in cooperation with the National Tuberculosis Association. In formulating such goals and standards the state health department should consult with its state tuberculosis association to develop a unified joint program of tuberculosis control for that state, taking into consideration the needs, extent of the problem, and resources of the various areas within the state. These state program blueprints should be made available to the U. S. Public Health Service and the National Tuberculosis Association to assist them in maintaining realistic national goals and standards.

   All such goals, program priorities, and performance standards should be reviewed and revised periodically to take cognizance of changing conditions and new knowledge.

   The Conference called attention to the article "Service Statistics for Tuberculosis Control Programs" published in *Public Health Reports* (Vol. 74, No. 4, April 1959), available from the U. S. Public Health Service; and the section on "Goals, Definitions and Performance Stand-

ards" in the *Highlights from the Report of the (1958–1959) NCTW Committee on Study of Program Activities*, available from the National Conference of Tuberculosis Workers, 1790 Broadway, New York 19, New York.

4. Periodic evaluation on a statewide basis of tuberculosis detection programs, with adjustment of such programs accordingly, so as to obtain maximum results from the funds available. The U. S. Public Health Service should provide assistance in such a study to any state that does not have the resources to conduct its own. The U. S. Public Health Service in cooperation with the National Tuberculosis Association should formulate and make available a guide for the conduct of such statewide evaluation of tuberculosis detection programs.
5. Periodic review by the U. S. Public Health Service of reporting practices within the various states in order to promote greater uniformity and to permit interpretation of comparable case report data.
6. Systematic local investigation of tuberculosis cases first reported by death certificate to be conducted by appropriate representatives of the local medical community.
7. Provision of laboratory services conveniently available to all physicians diagnosing and treating tuberculous patients. These services should meet certain minimum standards, such as:
   a) provision for the detection by culture, and the identification, of *Mycobacterium tuberculosis* and other strains of mycobacteria; and
   b) provision for performing tests of bacterial susceptibility to the antimicrobial agents most commonly used in the treatment of tuberculosis.

   The Conference also recommended that there be regularly recurring evaluation of the adequacy and accuracy of such laboratory services.
8. Use of BCG according to the recommendations made by the Ad Hoc Advisory Committee on BCG to the Surgeon General of the U. S. Public Health Service (AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES, Vol. 76, No. 5, November, 1957) and by the American Trudeau Society (*Ibid.*, Vol. 78, No. 1, July, 1958). The Conference further recommended that there be established improved standards for the evaluation of activity of BCG vaccines.
9. Intensification of research with regard to the social, psychological, and cultural factors that affect:
   a) acceptance of case detection and treatment, together with continuation of treatment;
   b) differential susceptibility of the individual to tuberculosis; and
   c) nature and results of treatment programs.

   This research would attempt to clarify what the social components are in the etiology of tuberculosis and in the program of tuberculosis control. The latter, in turn, would be concerned with the cultural and other social aspects of the population involved in the particular control program under consideration and with the social aspects of the manner in which the program is conducted (for example, how it deals with the so-called "recalcitrant" patient).
10. Intensification of research for a simple and accurate tuberculin test that can be applied and read by nonmedical personnel.
11. Recognition of the importance and potential significance of the current isoniazid prophylaxis field study of the Tuberculosis Program of the U. S. Public Health Service. The Conference recommended that adequate appropriations be continued by Congress for support until completion of this study, which may provide a significant shortcut to elimination of tuberculosis in this country.

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STUDIES ON THE METABOLISM OF ISONIAZID[1, 2]

I. Development and Application of a Fluorometric Procedure for Measuring Isoniazid in Blood Plasma

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With the technical assistance of R. Larsen and A. Tabri

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Introduction

Shortly after the introduction of isoniazid into the therapy of tuberculosis, the staff at this laboratory became actively interested in the metabolic fate of this drug. This interest led to characterization of the major products of the metabolism of isoniazid (1), to development of procedures for measuring parent compound and metabolites in urine (2), and to quantitative studies on the urinary excretion of these substances by experimental animals and man (3–5). The last-named of these investigations (5) indicated that human subjects varied widely in the extent to which they metabolized isoniazid. Some subjects possessed a high capacity to transform this hydrazide, eliminating less than 3 per cent of a given dose unchanged during twenty-four hours; others with lesser capacity excreted as much as thirty per cent as parent drug. In a given individual the pattern of metabolism appeared to be relatively constant and independent of the state of tuberculous disease. Since neither of the major metabolites of isoniazid, isonicotinic acid, or acetyl-isoniazid (1-acetyl-2-isonicotinylhydrazine) possessed antimycobacterial activity (6, 7), it was suggested (5) that these diverse patterns of metabolism might have a significant effect on the therapeutic efficacy of this drug. This suggestion was tested in a study on a group of 150 previously untreated patients with pulmonary tuberculosis. The specific objective was to relate the pattern of urinary excretion of isoniazid and its metabolites to rates of sputum "conversion," emergence of isoniazid-resistant bacilli, and cavity closure. This investigation yielded equivocal results (8, 9).

While the above study was in progress, other investigators became interested in the relationship between the metabolism of isoniazid and its therapeutic efficacy. Cognizant of the difficulties in quantitative collections of urine from patients, these workers focused their attention on the concentrations of isoniazid in serum (10–12). Utilizing microbiologic procedures for measuring the concentrations of active drug, these investigators have concluded that there is a direct relation between therapeutic response and the concentration of isoniazid in serum. It does seem that this conclusion has to be accepted with considerable reservation. The numbers of patients were relatively small, and multiple observations on individual subjects were extremely limited. Moreover, the large errors inherent in microbiologic procedures make it very difficult to appraise the significance of the comparatively small differences in concentrations of isoniazid observed frequently in these studies.

In the opinion of the present writer, studies on drug concentration–therapeutic effect relationships would be greatly facilitated by the availability of a specific, highly sensitive, reliable chemical procedure for measuring isoniazid in plasma or serum. Existing chemical methods are unsatisfactory for this purpose. Thus, the procedures of Short (13) and Scardi (14) are not sufficiently sensitive, while the methods of Hunter (15) and of Poole and Meyer (16) have limited utility because of the high blanks of drug-free serum. The procedure of Maher and associates (17) suffers from a lack of reproducibility at isoniazid concentrations of 1 γ or less per ml. of plasma or serum.

Numerous attempts by the present writer and Hughes of this laboratory to develop a suitable colorimetric procedure were unsuccessful. Subsequent efforts led to the fluorometric procedure here described. This method, which is based on the production of a fluorescent derivative of

[1] From The Christ Hospital Institute of Medical Research, Cincinnati, Ohio.

[2] A preliminary account of these studies was presented at the Eighteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration-Armed Forces, February, 1959.

isoniazid by reaction with cyanogen bromide, is an adaptation of the procedure of Chaudhuri and Kodicek (18) for measuring nicotinamide. The method is extremely sensitive, yet has sufficient flexibility to permit measurement of isoniazid over a range of concentrations from 0.1 to 10 γ per ml. of plasma or serum.

The present report deals with a description of the procedure and of its application to the measurement of the concentration of isoniazid in the plasma of rats, dogs, monkeys, and human volunteers treated with this compound. In order to place the data acquired with this fluorometric method in proper perspective, simultaneous assays have been made of the concentrations of total hydrazides in plasma. In the studies with human volunteers, measurements of the concentrations of isoniazid and total hydrazides in plasma and of the urinary elimination of these substances have been made concurrently.

Experimental Methods

Fluorometric determination of isoniazid: As mentioned already, the assay technique for isoniazid was based on the method of Chaudhuri and Kodicek (18) for estimating nicotinamide. Radical departures from the original conditions in regard to type of buffer, pH of reaction with cyanogen bromide, and time and temperature for development of the fluorescent complex resulted in the production of a fluorescent derivative of isoniazid and the elimination of the usual fluorescence contribution of nicotinamide. The addition of ethanol to the final reaction mixture gave a two-fold enhancement of the fluorescence contributed by isoniazid.

Isoniazid was isolated from endogenous fluorescent substances in plasma by employing, with modifications, a double-extraction procedure described previously (19). This technique also extracts acetyl-isoniazid. Since this metabolite does not yield a fluorescent product by reaction with cyanogen bromide, it was possible to measure isoniazid fluorometrically and total hydrazides (the sum of isoniazid and acetyl-isoniazid) colorimetrically in a single plasma sample by taking appropriate aliquots of the final acid extract.

The procedure for analysis of isoniazid in plasma was as follows: Three milliliters of citrated plasma[3] were mixed with 3.2 gm. of ammonium sulfate[4] in a 60-ml. glass-stoppered bottle, following which 1.0 ml. of 0.10 N sodium hydroxide and 40 ml. of a 3:7 isoamyl alcohol-ethylene dichloride mixture[5] were added. After shaking in a horizontal position for thirty minutes, the extraction mixture was transferred to a heavy-walled tube, and centrifuged at 1,500 r.p.m. for fifteen minutes. Thirty milliliters of the organic solvent phase were then delivered into a 25 × 150-mm. screwcap (Teflon liner) culture tube containing 5 ml. of alkalinized ammonium sulfate solution,[6] pH 7.8. This mixture was shaken for fifteen minutes and centrifuged at 1,500 r.p.m. for ten minutes, after which 25 ml. of the solvent phase were transferred to another culture tube containing 4.0 ml. of 0.10 N hydrochloric acid. This tube and contents were shaken for fifteen minutes and centrifuged for five minutes at 1,500 r.p.m., yielding a 0.1 N hydrochloric acid solution of isoniazid and acetyl-isoniazid.

A 2-ml. aliquot of the acid extract was transferred to a 15-× 150-mm. tube. It was treated with 1.0 ml. of 0.20 N sodium hydroxide, 0.5 ml. of ammoniacal buffer, pH 8.3 (20), 0.5 ml. of 5 per cent aqueous cyanogen bromide[7] (fume hood), 0.5 ml. of 1.8 N carbonate-free sodium hydroxide, and 2.0 ml. of triple-distilled ethanol, with vigorous shaking after each addition. The reaction mixture was transferred to a 10-× 100-mm. quartz tube. Intensity of fluorescence was measured with an Aminco-Bowman spectrophotofluorometer[8] using slit arrangement 4 and a 1P21

[3] Less plasma may be used but the volume should be adjusted to 3 ml. with distilled water. The range of concentrations of isoniazid can be extended to 30 γ per ml. by taking 1 ml. of plasma for assay.

[4] Baker and Adamson, Reagent Special, pyridine-free.

[5] Commercial i-amyl alcohol (Matheson, Coleman and Bell, A.C.S. Reagent) was purified by washing 2 parts of the alcohol successively with 1 part of 2.5 N hydrochloric acid, 1 part of 2.5 N sodium hydroxide and twice with equal parts of water. The alcohol was then dried with three separate portions of anhydrous potassium carbonate (Mallinckrodt, A. R.) (50 gm. per liter), allowing the solvent to dry overnight after addition of the third portion. After filtration, the i-amyl alcohol was fractionally distilled, collecting the fraction boiling at 131–132° C. which exhibited optical densities of less than 0.020 at 300 mμ and 0.200 at 270 mμ against 0.10 N hydrochloric acid. Ethylene dichloride (Matheson, Coleman and Bell, Reagent Grade) was purified by treating 4 liters with 10 gm. of decolorizing charcoal and allowing to stand overnight. After filtration, the ethylene dichloride was fractionally distilled, collecting the water-free fraction boiling at 83–84° C.

[6] One liter of 70 per cent ammonium sulfate (pyridine-free) is treated with 2.5 ml. of 18 N sodium hydroxide.

[7] Eastman, Reagent Grade. Prepare fresh solution at weekly intervals.

[8] American Instrument Co., Inc., Silver Spring, Maryland. The instrument used was calibrated as previously described (21). Quinine in 0.1 N sulfuric acid exhibited an activation maximum of 355 mμ and a fluorescence maximum of 460 mμ. The former value is within 5 mμ of that obtained

phototube. The instrument was set at the maximum activation wavelength, 300 mμ, and the maximum fluorescence wavelength, 405 mμ, of the isoniazid-cyanogen bromide reaction product. The fluorescent complex was stable during one hour.

The fluorescence intensities[9] obtained when various amounts of pure isoniazid[10] in 0.1 N hydrochloric acid were reacted with cyanogen bromide, as described above, are summarized in figure 1. The data therein indicate the high sensitivity and the broad range of linearity in the analysis of pure solutions. It must be emphasized that, to acquire data of the reliability indicated in this figure, it is necessary to compensate continually for fluctuations in the intensity of the xenon arc lamp of the spectrophotofluorometer. This has been accomplished by use of a standard sample which contains 2 γ of isoniazid. Using this standard, the instrument was adjusted[11] to an arbitrarily selected point of 50 per cent transmission (meter multiplier at 0.3) at the beginning of a series of readings. Instrument variation was then controlled by readjusting to this point frequently in a series of assays by use of the same standard sample.

When isoniazid was added to plasma of rat, dog, rhesus monkey, or man in concentrations of 0.1 to 10 γ per ml. and the samples were subjected to the extraction and fluorometric procedures, recovery curves were obtained which were linear and practically identical in all species. However, the absolute fluorescence intensities were systematically 20 per cent lower than those of the same concentrations of isoniazid in 0.1 N hydrochloric acid not extracted. These lower fluorescence intensities have been shown to be independent of the presence of plasma. Instead, they were due to quenching of fluorescence by components of the extraction system. When isoniazid extracted from aqueous solution was used as a reference standard, recovery of the drug from plasma ranged from 95 to 105 per cent.

Under the conditions employed in the fluorometric procedure, acetyl-isoniazid[12] and isonicotinic acid do not interfere with the analysis. Plasma samples containing as much as 30 γ of these substances per ml. yielded fluorescence intensities corresponding to less than 0.1 γ of isoniazid. In addition, plasma samples from untreated subjects, when analyzed as described above, gave fluorescence intensities corresponding to less than 0.1 γ of isoniazid per ml.

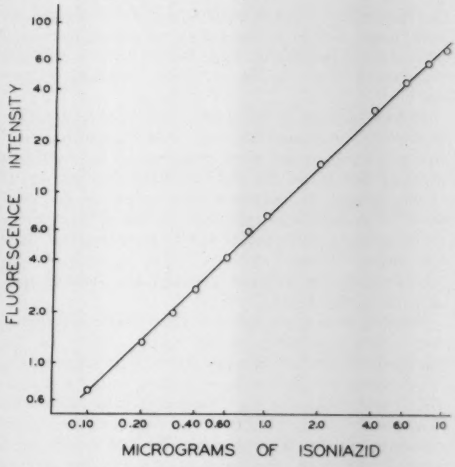

FIG. 1. Relationship between isoniazid content of the assay solution and fluorescence intensity of the isoniazid-cyanogen bromide reaction product.

Colorimetric determination of total hydrazides: Total hydrazides were determined by minor modifications of the Kelly and Poet method (23) as adapted by Schmidt and associates (19). One milliliter of the acid extract described in the above section was placed in a 10-ml. graduated test tube to which 1 ml. of 3 N hydrochloric acid and 1 ml. of the *p*-dimethylaminobenzaldehyde color reagent were added. The tube was then fitted with a glass bubble condenser, heated for one hour in a boiling water bath, cooled to room temperature in an ice bath, and the volume was readjusted to 3 ml. with 0.1 N hydrochloric acid. Color intensity was measured in a micro-colorimeter tube on a Klett-Summerson colorimeter equipped with a No. 44 filter. The resultant reading was referred to values obtained with standard isoniazid solutions and was expressed as isoniazid equivalents. Both isoniazid and acetyl-isoniazid give stoichiometric quantities of hydrazine in

by the previous writers and within the limit of precision on the instrument used. However, the fluorescence maximum of 460 mμ should be corrected to 450 mμ. Thus, the corrected value of the fluorescence maximum of the isoniazid-cyanogen bromide reaction product would then be 395 mμ.

[9] Fluorescence intensity is the product of the per cent transmission read from the photometer and the meter multiplier setting.

[10] The isoniazid used in these studies was recrystallized from *n*-butanol using decolorizing charcoal. After drying *in vacuo* for several hours at 45° C., it exhibited a melting point of 170–171°C. and a molar extinction coefficient of 5,000 at 266 mμ in 0.1 N hydrochloric acid (22). These data indicate a purity of 98–100 per cent.

[11] This adjustment was made by use of the variable photometer sensitivity.

[12] The acetyl-isoniazid (1-acetyl-2-isonicotinylhydrazine) used in this work had a melting point of 158–159°C. It exhibited a molar extinction coefficient of 6,300 at 265 mμ in 0.1 N hydrochloric acid (1). These data indicate a purity of 98–100 per cent.

the procedure. Systematic studies of plasma of rats, dogs, monkeys, and man to which isoniazid and acetyl-isoniazid had been added yielded recoveries of 91 to 96 per cent, average 94 per cent.

Determination of isoniazid and total hydrazides in urine: The concentrations of isoniazid and total hydrazides in urine were determined by methods already described (2). As indicated previously, it is necessary to measure the amounts of non-specific materials eliminated by the subject prior to treatment and correct the post-treatment data for these "blank" values. This procedure was followed in the urinary elimination studies presented in this report.

Studies on various subjects treated with isoniazid: As a demonstration of the applicability of the methods described, studies have been made of the concentrations of the parent drug and its hydrazide metabolites in the plasma of rats, monkeys, dogs, and human volunteers at various times after administration of isoniazid. In the work with human subjects, the urinary elimination of the above substances has been studied simultaneously.

Female Sprague-Dawley rats (200 ± 10 gm.), male and female beagle dogs (8.5 to 11.5 kg.), and male and female rhesus monkeys (7.6 to 10.6 kg.) were used in these experiments. All animals were maintained on stock diets and allowed water *ad libitum* during the experimental periods. Isoniazid[10] was administered intramuscularly to the rats and dogs and both intramuscularly and intravenously to the monkeys at doses ranging from 5 to 20 mg. per kg. of body weight. Blood samples were obtained prior to, and one, two, four, and eight hours after treatment. Four to eight rats were bled from the heart at each of these periods, 5 ml. of blood being obtained from each animal. In the studies on dogs and monkeys, blood samples were withdrawn serially from individual animals. Ten-milliliter quantities were obtained at each venipuncture. In all of the studies, 2.5 per cent sodium citrate (1 part to 5 parts of blood) was used as the anticoagulant.

Ten human volunteers, all males, ranging in age from twenty-two to forty-nine years and in weight from 67.6 to 96.8 kg., were used in this study. None were receiving other medication during the experimental periods. Isoniazid[10] was administered orally in single doses of 5 or 10 mg. per kg. In each case, the appropriate amount of drug was dissolved in 250 ml. of water. Ten milliliters of venous blood were withdrawn from each of these subjects one, two, four, and eight hours after ingestion of isoniazid. Plasma samples for blank determinations were obtained one day before and two days after treatment. Urine was collected at intervals corresponding to the times of blood assays and, in addition, during the eight- to sixteen- and sixteen- to twenty-four-hour post-treatment periods. In order to ensure sufficient urine volumes at the earlier periods, 250 ml. of water were ingested one hour post treatment. All urine specimens were refrigerated under toluene prior to analysis.

Results

The observations on the concentrations of isoniazid and its hydrazide metabolites in the plasma of rats, dogs, and rhesus monkeys following isoniazid administration have been summarized in table 1 and figures 2, 3, and 4. It is immediately apparent from the small standard deviations in these data that there was remarkable uniformity in the reactions of individual members of these species. It is equally clear that the disposition of isoniazid in rats and monkeys was strikingly different from that in dogs.

As shown in table 1 and figures 2 and 3, there was a precipitous decrease in the concentrations of unchanged isoniazid in the plasma of rats which received the drug intramuscularly and of monkeys subjected to either intramuscular or intravenous administration. At the 20 mg. per kg. dosage level, the concentration of isoniazid in rat plasma fell from 12 γ per ml. at one hour to 3.5 γ per ml. at two hours, and to less than 0.1 γ per ml. at four hours (figure 2). The concentrations in the plasma of monkeys at the same dose at comparable times were 12, 6.5, and 1.8 γ per ml. (figure 3). In both of these species, the concentration of hydrazide-containing substances in plasma one or more hours after administration greatly exceeded the concentration of parent drug. These differences increased with time. Comparison of the data of table 1 with figure 3 indicates that the route of administration had little effect on the disposition of isoniazid in the monkey. These observations in the studies using rats and monkeys point quite clearly to rapid and continuous conversion of isoniazid to other hydrazide metabolites and suggest that this

TABLE 1

Plasma Concentrations of Isoniazid and Total Hydrazides in Monkeys Receiving Isoniazid in Doses of 5 Mg. per Kg. Intramuscularly

Time After Administration	Concentration (γ/ml.) in Plasma*	
	Isoniazid Mean ± Standard Deviation	Total Hydrazides Mean ± Standard Deviation
hours		
1	3.3 ± 0.3	5.2 ± 0.4
2	1.5 ± 0.2	3.9 ± 0.3
4	0.6 ± 0.2	2.8 ± 0.3
8	0.0	1.1 ± 0.2

* The values are means of 6 animals.

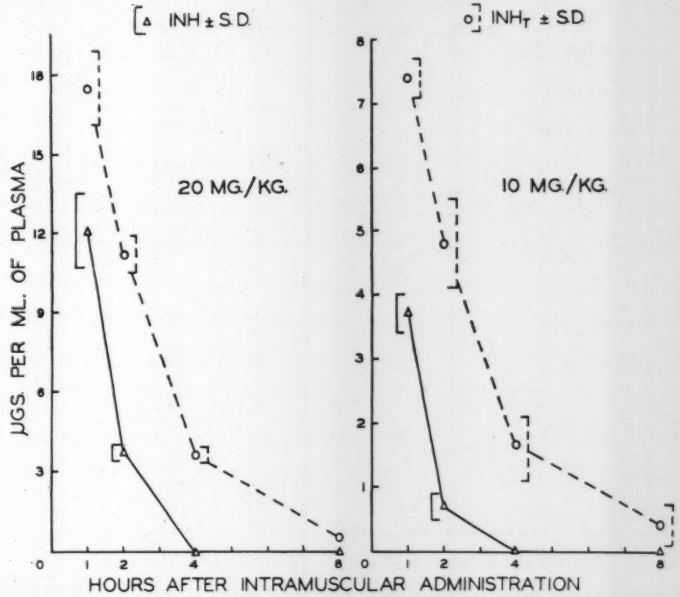

FIG. 2. Mean plasma concentrations of isoniazid (INH) and total hydrazides (INH$_T$) in rats receiving isoniazid in doses of 10 and 20 mg. per kg., intramuscularly. At the lower dose, each point is the mean of 8 rats; at the higher dose each point is the mean of 4 rats.

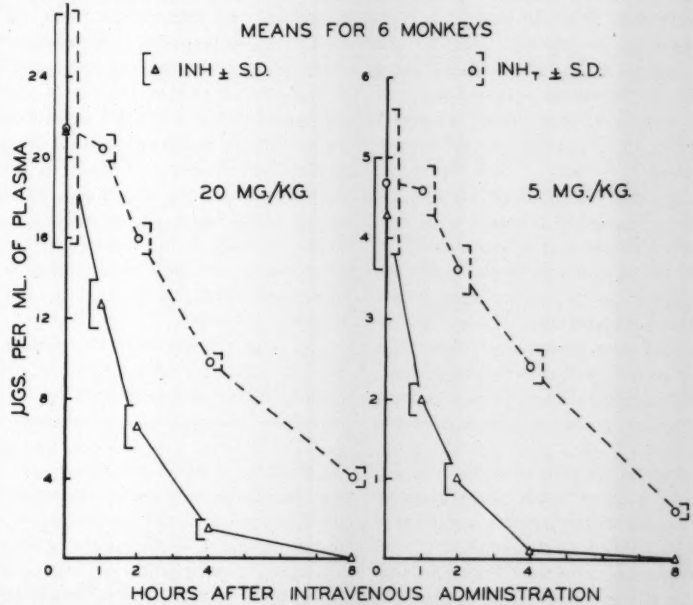

FIG. 3. Mean plasma concentrations of isoniazid (INH) and total hydrazides (INH$_T$) in monkeys receiving isoniazid in doses of 5 or 20 mg. per kg., intravenously. Zero-hour values were obtained from blood taken 2 to 3 minutes after injection.

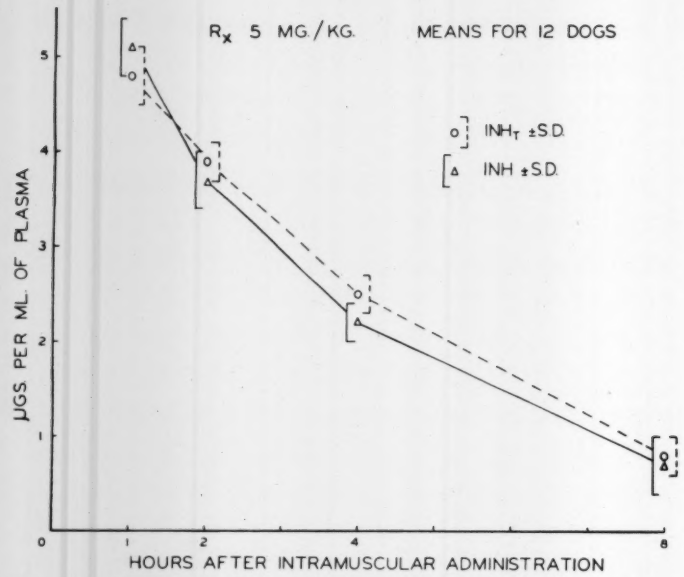

FIG. 4. Mean plasma concentrations of isoniazid (INH) and total hydrazides (INH_T) in dogs receiving isoniazid in doses of 5 mg. per kg. intramuscularly.

reaction is one of prime importance in the removal of parent drug from the blood.

The above findings in rats and monkeys are in interesting contrast to the observations using dogs (figure 4). In this latter species, the concentrations of unchanged drug greatly exceeded the concentrations found in monkeys under comparable conditions (*cf.* table 1 and figure 4). Furthermore, isoniazid disappeared at a much slower rate in dogs than in either rats or monkeys. Thus, in figure 4 a decrease is shown from 5.1 γ of isoniazid per ml. at one hour to 2.3 γ per ml. at four hours and 0.7 γ per ml. at eight hours. At all times, the concentrations of total hydrazides and isoniazid were essentially equal. This finding can only be interpreted as indicating that, unlike rats and monkeys, dogs do not convert isoniazid to other hydrazide-containing metabolites. The maintenance of higher concentrations of isoniazid in dogs, at the present state of knowledge, seems to be a direct result of the inability of this species to convert the parent drug to other hydrazides, presumably acetyl-isoniazid.

The data on human volunteers (figure 5) suggest that, insofar as the conversion of isoniazid to other hydrazides is concerned, men resemble rats and monkeys rather than dogs. However, the magnitudes of the standard deviations show that there were tremendous variations in the concentrations of isoniazid in the plasma of different subjects. For this reason, attention is directed to the detailed data of table 2. It may be seen in columns 2 to 5 of this table that at the 5 mg. per kg. dose there was as much as a fourfold variation in the plasma concentrations of different subjects one hour after drug ingestion, a sixfold variation at two hours, and a thirteen fold spread at four hours. At eight hours, drug concentrations were at or below the limit of detection in 3 subjects and varied from 0.2 to 1.4 γ per ml. in the remaining subjects.

The wide deviations in the plasma concentrations of isoniazid encountered in different subjects are illustrated graphically in figure 6, which compares observations on subjects *A. G.* and *J. P.* at doses of 5 and 10 mg. per kg. Three aspects of the data in this figure are noteworthy. The first is the rapid absorption followed by the precipitous fall in the concentration of isoniazid in the plasma of *A. G.* compared with the slower absorption and gradual decline of plasma concentrations in *J. P.* The second is the similarity of the plasma decay curves of each subject at the two doses, suggesting that those factors causing

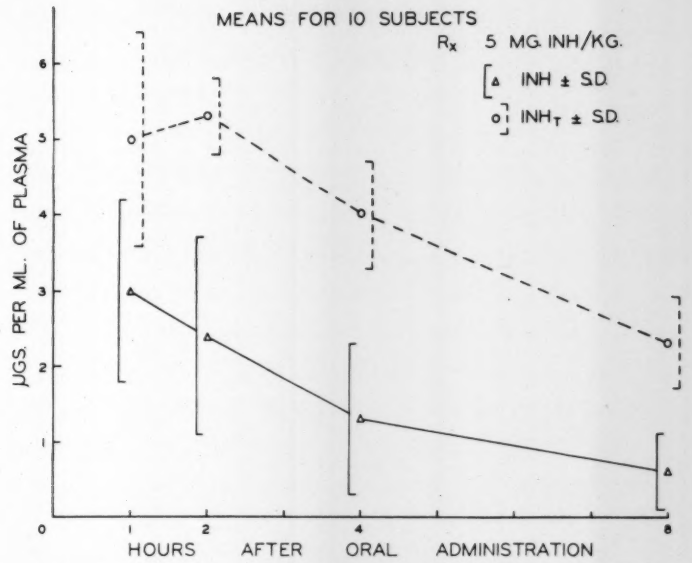

FIG. 5. Mean plasma concentrations of isoniazid (INH) and total hydrazides (INH_T) in human subjects receiving isoniazid in doses of 5 mg. per kg., orally.

TABLE 2

PLASMA CONCENTRATIONS AND URINARY EXCRETION OF ISONIAZID IN HUMAN SUBJECTS RECEIVING ISONIAZID ORALLY

Subject	Plasma Concentration (γ/ml.)				Urinary Excretion (per cent of dose)				
	1 hour	2 hours	4 hours	8 hours	0–2 hours	2–4 hours	4–8 hours	8–24 hours	0–24 hours
After 5 mg./kg.									
G. B.	1.9	0.7	0.2	0.0	4.0	0.9	0.5	0.1	5.5
A. G.	1.9	0.7	0.3	0.1	3.2	0.9	0.0	0.0	4.1
L. S.	2.3	1.3	0.4	0.1	2.9	1.6	0.4	0.0	4.9
J. L.	2.8	1.8	0.6	0.2	4.2	2.5	0.7	0.6*	8.0*
R. S.	4.1	2.2	0.7	0.3	4.5	2.1	1.0	0.0	7.6
J. McB.	3.0	2.8	1.9	0.7	5.0	4.4	4.2	3.4	17.0
J. K.	4.8	3.1	2.0	1.0	8.7	5.5	2.2	1.9	18.3
J. P.	3.4	3.8	2.4	1.1	4.2	5.5	5.4	5.2	20.3
A. T.	1.2	3.9	2.7	1.3	1.6	3.9	5.4	4.0	14.9
R. H.	4.4	4.0	2.2	1.4	6.2	5.2	5.8	1.7	18.9
After 10 mg./kg.									
A. G.	4.5	2.8	0.7	0.0	3.5	1.3	0.1	0.0	4.9
R. S.	7.4	4.9	1.9	0.4	5.0	3.1	1.9	0.8	10.8
J. K.	7.5	7.4	4.9	2.4	5.3	4.3	3.0	3.0	15.6
J. P.	5.5	8.0	5.6	2.5	5.4	5.0	5.0	8.4	23.8
A. T.	3.4	8.4	5.9	2.6	2.6	3.3	5.6	7.1	18.6

* In this series, the 16- to 24-hour urine fraction was lost. Therefore these values are the 8- to 16-hour and 0- to 16-hour fractions, respectively.

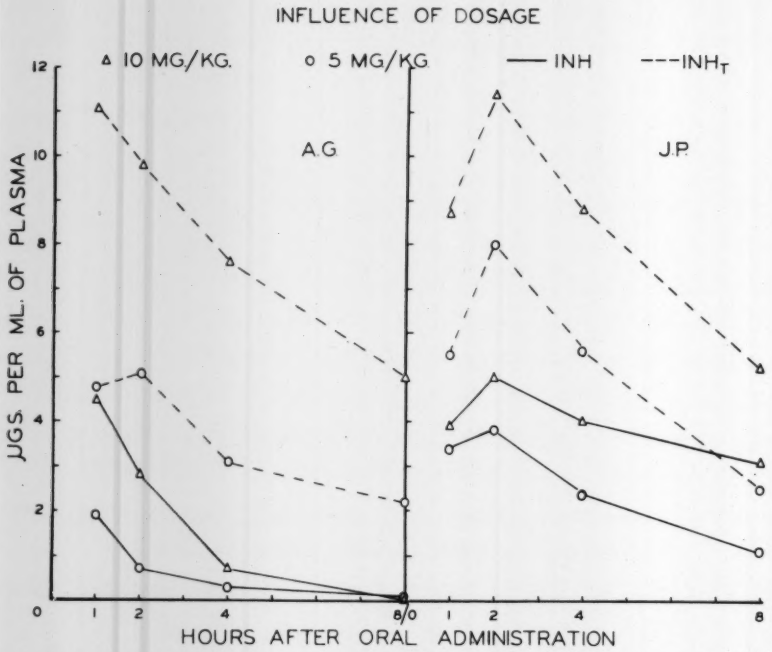

FIG. 6. The influence of dosage on the plasma concentrations of isoniazid (INH) and total hydrazides (INH_T) in 2 male human subjects.

the disappearance of isoniazid from plasma are independent of dosage. The third is the relative similarity of the total hydrazide curves in both subjects in contrast to the diverse patterns of the isoniazid curves.

The variations in the concentrations of total hydrazides in plasma were relatively small in comparison with the differences in isoniazid concentrations just discussed. Thus, as shown in columns 2 to 5 of table 3, there was less than a twofold variation in the concentrations of these moieties two and four hours after ingestion of the drug. At eight hours, the differences among individuals were less than threefold. This finding assumes special importance when correlated with the results of the urinary studies.

The relations between the plasma concentrations of isoniazid and total hydrazides and the urinary elimination of these moieties are a matter of considerable interest inasmuch as the urinary output more accurately reflects the ultimate fate of the drug. The measurements of the urinary elimination of isoniazid made concurrently with the plasma assays are presented in columns 6 to 9 of table 2. For convenience of presentation and examination, the results of the zero- to one- and one- to two-hour collections have been combined as have the data on the eight- to sixteen- and sixteen- to twenty-four-hour fractions. A comparison of plasma concentrations and urinary excretion shows that there is an excellent correlation between the concentration of isoniazid in the general circulation and the urinary elimination of this compound. Those subjects who had the lowest concentrations of parent drug in plasma excreted the least amount, whereas those exhibiting higher plasma concentrations eliminated larger fractions of their dose in the urine. This correlation is apparent even in those subjects, *J. P.* and *A. T.*, who exhibited a slower rate of absorption of isoniazid than the other subjects. The absolute differences in total urinary excretion (column 10) were considerable, ranging from 4.1 to 20.3 per cent when a dose of 5 mg. per kg. was administered. The results obtained in those subjects also receiving doses of 10 mg.

TABLE 3

PLASMA CONCENTRATIONS AND URINARY EXCRETION OF TOTAL HYDRAZIDES IN HUMAN SUBJECTS RECEIVING ISONIAZID ORALLY

Subject	Plasma Concentration (γ/ml.)				Urinary Excretion (per cent of dose)				
	1 hour	2 hours	4 hours	8 hours	0–2 hours	2–4 hours	4–8 hours	8–24 hours	0–24 hours
After 5 mg./kg.									
G. B.	5.5	4.9	2.7	1.3	19.9	14.3	20.8	11.8	66.8
A. G.	4.8	5.1	3.2	2.4	14.1	10.1	19.6	19.0	62.8
L. S.	6.3	5.9	4.7	2.2	12.2	11.2	16.1	13.2	52.7
J. L.	5.8	5.8	3.9	2.0	13.6	12.9	18.0	15.8*	60.3*
R. S.	6.4	5.4	4.8	2.4	12.7	11.0	15.4	25.8	64.9
J. McB.	4.7	4.7	3.6	1.5	9.7	9.7	14.3	17.2	50.9
J. K.	5.7	5.2	3.9	2.4	12.5	10.8	8.9	14.8	47.0
J. P.	3.9	5.0	4.0	3.1	6.7	10.4	15.0	21.9	54.0
A. T.	1.6	4.9	4.4	2.5	3.8	9.7	17.5	24.7	55.7
R. H.	5.6	6.2	4.6	3.0	8.4	9.5	13.0	15.5	46.4
After 10 mg./kg.									
A. G.	11.1	9.8	7.6	5.0	10.5	10.0	18.0	20.3	58.8
R. S.	12.9	10.5	8.0	5.1	10.5	10.3	15.6	21.9	58.3
J. K.	11.7	11.0	8.1	5.5	8.7	9.8	8.5	16.6	43.6
J. P.	8.7	11.4	8.8	5.4	6.4	8.3	8.4	23.8	46.9
A. T.	4.8	10.5	9.3	5.7	4.0	7.3	15.2	27.5	54.0

* In this series, the 16- to 24-hour urine fraction was lost. Therefore, these values are the 8- to 16-hour and the 0- to 16-hour fractions, respectively.

per kg. followed the patterns exhibited at the lower dosage level.

As shown in columns 6 to 10 of table 3, the urinary elimination of total hydrazides, like the concentrations of these materials in plasma, fell within narrow limits. During twenty-four hours, between 46.4 and 66.8 per cent of the drug administered at 5 mg. per kg. and between 43.6 and 58.8 per cent at 10 mg. per kg. were eliminated. Again, an excellent correlation between plasma concentrations and urinary excretion of these moieties was observed.

DISCUSSION

The question of whether a method measures specifically that material for which it was designed is of utmost importance in evaluating a new analytic procedure. The following facts indicate that the procedure described does measure isoniazid. Blank values of plasma from untreated animals or humans were low. The material being measured has the solubility characteristics of isoniazid, and recoveries of parent drug added to plasma were reproducible. Frequent checks on extracts have shown that the reaction with cyanogen bromide was necessary to produce a material capable of fluorescence. In experimental extracts, the characteristics of fluorescence, i.e., maximal activation and fluorescence wavelengths, were identical to those of the isoniazid-cyanogen bromide complex in pure solution. Finally, the major metabolites of isoniazid, acetyl-isoniazid, and isonicotinic acid did not interfere with or contribute to the fluorescence obtained with isoniazid.

It should be mentioned that two possible metabolites of isoniazid, the pyruvic acid and α-ketoglutaric acid isonicotinyl hydrazones, would be measured as parent drug in this procedure. These compounds are split completely to isoniazid by exposure to 0.1 N hydrochloric acid or when their aqueous solutions are extracted as described for plasma (24). Although they have not been detected in plasma of animals or humans receiving isoniazid, Zamboni and Defranceschi (25) have presented chromatographic evidence of their presence in rat urine following administration of 100 mg. of isoniazid per kg. By the same

TABLE 4

APPROXIMATE BIOLOGIC HALF-LIFE OF ISONIAZID IN THE PLASMA OF RATS, MONKEYS, DOGS, AND HUMANS

Species	Dose	Route of Administration	Approximate Biologic Half-Life
	mg./kg.		*hours*
Rat	10	Intramuscular	0.4
	20	Intramuscular	0.6
	25	Intraperitoneal	0.4*
Monkey	5	Intramuscular	1.2
	5	Intravenous	0.9
	20	Intravenous	1.0
Dog	5	Intramuscular	2.5
Man			
G. B.	5	Oral†	1.0
A. G.	5	Oral	1.1
	10	Oral	1.1
L. S.	5	Oral	1.6
J. L.	5	Oral	1.9
R. S.	5	Oral	1.6
	10	Oral	1.7
J. McB.	5	Oral	3.1
J. K.	5	Oral	3.7
	10	Oral	3.6
J. P.	5	Oral	3.4
	10	Oral	3.6
A. T.	5	Oral	3.9
	10	Oral	3.6
R. H.	5	Oral	4.0

* Estimated from data of Ceriotti *et al.* (28). Isoniazid was measured microbiologically.

† Estimated using only the 2-, 4-, and 8-hour assay values.

technique, Iwainsky (26) has reported their occurrence in the urine of humans who received 300 mg. of isoniazid. Furthermore, previous work in this laboratory (3, 5) has shown that the urine of rats, dogs, monkeys, and humans to whom isoniazid has been administered contains hydrazone-like substances. Therefore, these acid-labile urinary metabolites must be considered, at the present, as possible contributors to the isoniazid measured fluorometrically.

The differences between isoniazid measured fluorometrically and total hydrazides measured colorimetrically in plasma are undoubtedly due to the metabolite, acetyl-isoniazid. The presence of this derivative in the plasma of monkeys and humans receiving isoniazid has already been demonstrated in this laboratory (3). In addition, it has been found in the plasma and liver of rats administered carbon-14 labeled isoniazid (27).

In order to relate numerically the metabolic response of the various species, graphic estimations of the biologic half-lives of isoniazid in plasma have been made. These values are presented in table 4. It is immediately apparent that the rat was the most active in disposing of isoniazid. The rhesus monkey, while exhibiting a plasma disappearance pattern very similar to that of the rat (figures 2 and 3), was, however, approximately one-half as active in disposing of isoniazid, as shown by the half-life values. The dog, on the other hand, apparently reflected its inability to acetylate isoniazid (figure 4) by the comparatively slow half-time disappearance of 2.5 hours. In estimating these values in man, the influence of variations in absorption following oral administration was eliminated by omitting the one-hour plasma concentrations. The resulting half-lives (table 4) show that humans exhibited an extremely broad range of capabilities for disposition of the drug.

At the dosages studied, the values of table 4 confirm that the response of the animal or individual was independent of the amount of drug administered. In addition, the values derived from the monkey experiments suggest that the route of administration was without marked influence. It is evident that absorption from the site of intramuscular injection was extremely rapid.

In general, there is good agreement between the present results and those obtained previously by others. Thus, as may be seen in table 4, estimation of the biologic half-life of isoniazid in the rat from data of Ceriotti and associates (28) resulted in a value of 0.4 even though the dose, route of administration, and method of assay were different. It may be seen in figure 2 that, shortly after administration, the concentrations of total hydrazides in rat plasma greatly exceeded the concentrations of unchanged isoniazid. The difference in these moieties, which measures the extent of acetylation, indicates that the acetyl derivative was present in quantities three to five times greater than the parent drug. These observations are compatible with the previous qualitative demonstration of the presence of isoniazid and acetyl-isoniazid in rat blood after administration of the drug (27). In addition, they agree essentially with the observations of Hughes and Schmidt (3) that the rat eliminated in the urine 6 to 10 per cent of administered isoniazid unchanged and 40 to 50 per cent as acetyl-isoniazid.

The absence of hydrazide-containing materials other than isoniazid in the dog blood provides confirmation of earlier findings that the dog is incapable of acetylating isoniazid (3, 29). Thus, Hughes and Schmidt (3) found no acetyl-isoniazid and 38 to 52 per cent of the unchanged drug in dog urine after administration of isoniazid.

In a similar manner, the rapid and extensive acetylation by the monkey evidenced here (figure 3) is compatible with the results of earlier studies in urine and blood (1, 3). Likewise, the data on human volunteers confirm and re-emphasize previous conclusions derived from urinary elimination studies. These earlier studies had shown that the output of total hydrazides was relatively similar in different subjects, but that there were wide variations in the urinary elimination of isoniazid, "isoniazid hydrazones," and acetyl-isoniazid. In general, there was a reciprocal relation between the proportion of unchanged isoniazid and acetyl-isoniazid excreted (5). Indirectly, this same relationship can be deduced from the results of the current studies on both blood and urine in which the differences between the concentrations or percentage of total hydrazides and unchanged isoniazid represent acetyl-isoniazid. Thus, it is clear from the data in tables 2 and 3 that those subjects who had the highest plasma concentrations of isoniazid had the lowest levels of acetyl-isoniazid, and vice versa. This finding implies that the capacity of the individual to acetylate isoniazid is of prime importance in determining the concentration of unchanged drug in plasma and the proportion of isoniazid found in urine.

The excellent correlation between plasma concentrations and urinary elimination as shown in tables 2 and 3 suggests that plasma concentrations do, indeed, reflect the ultimate fate of the drug. This correlation also seems to eliminate differences in renal excretion as a factor contributing to variations in the amount of isoniazid in the blood among different subjects.

Summary

A fluorometric method for the measurement of isoniazid in plasma, sensitive to 0.1 γ per ml. and reproducible to the extent of ± 5 per cent, has been described. This procedure has been applied to measurements of the concentrations of this drug in the plasma of rats, monkeys, dogs, and men at various times after drug administration. Total hydrazides in plasma have been determined simultaneously. These studies have shown that isoniazid disappears very rapidly from the plasma of the rat and the monkey, and indicate that a large fraction of the drug is converted to acetyl-isoniazid. In contrast, isoniazid is cleared relatively slowly from the plasma of the dog and is not converted to acetyl-isoniazid by this species. Human subjects vary widely in the ability to dispose of isoniazid; however, for a given individual, the pattern of disposition is essentially the same at doses of 5 and 10 mg. of isoniazid per kg. The correlation between plasma concentrations and urinary excretion of isoniazid and total hydrazides in the human subject was excellent. It has been suggested that, in both animals and man, the differences observed in the magnitude and maintenance of isoniazid in the plasma are more dependent upon variations in acetylating capacities than upon dissimilar renal excretion.

Sumario

Estudios del Metabolismo de la Isoniacida. I. Elaboración y Aplicación de un Procedimiento Fluorométrico para Medir la Isoniacida en el Plasma Sanguíneo

Descríbese un método fluorométrico para la medición de la isoniacida en el plasma, susceptible a 0.1 γ por ml. y reproducible hasta ± 5 por ciento. Se ha aplicado este procedimiento a mediciones de las concentraciones de dicha droga en el plasma de ratas, monos, perros y personas a varios plazos después de la administración de la droga. Se determinaron simultáneamente las hidracidas totales en el plasma. Demostraron estos estudios que la isoniacida desaparece muy rápidamente del plasma de la rata y del mono e indican que una gran fracción de la droga se convierte en isoniacida acetílica. En contraposición, la isoniacida desaparece con relativa lentitud del plasma del perro y no la convierte en isoniacida acetílica esta especie. Los sujetos humanos varían sumamente en su capacidad para disponer de la isoniacida; sin embargo, para un sujeto dado, el patrón de la disposición es en el fondo idéntico a dosis de 5 y 10 mgs. de isoniacida por kg. de peso. Fué excelente la correlación entre las concentraciones en el plasma y la excreción urinaria de isoniacidas e hidracidas totales en el sujeto humano. Se apunta que, tanto en los animales como en el hombre, las diferencias observadas en la magnitud y el mantenimiento de las concentraciones de isoniacida en el plasma dependen más de las variaciones en la capacidad acetiladora que de la distinta excreción renal.

Resume

Etude sur le métabolisme de l'isoniazide. I. Développement et application d'un procédé fluoromètrique de mesure de l'isoniazide dans le plasma sanguin

L'auteur décrit une méthode fluoromètrique de mesure de l'isoniazide dans le plasma, sensible à 0,1γ par ml. et allant jusqu'à 5%. Ce procédé a été appliqué à la mesure de la concentration de ce médicament dans le plasma de rats, de singes, de chiens et d'hommes, à des moments différents après l'administration du médicament. On a déterminé en même temps les hydrazides totales dans le plasma. Ces études ont montré que l'isoniazide disparait trés rapidement du plasma du rat et du singe, et montre qu'une large proportion du médicament est transformée en acétyl-isoniazide. Au contraire, l'isoniazide est éliminée plutôt lentement du plasma du chien et n'est pas transformée en acétyl-isoniazide par cette espèce. Les sujets humains varient largement dans leur possibilité de métaboliser l'isoniazide; cependant pour un sujet donné, le mode de métabolisme est essentiellement le même à des doses de 5 et 10 mg. d'isoniazide par kg. Il y a une excellente corrélation entre les concentrations dans le plasma, la quantité d'isoniazide excrétée dans l'urine et les hydrazides totales chez l'homme. Il a été suggéré que chez les animaux et chez l'homme, les différences observées dans la quantité et la régularité des concentrations d'isoniazide dans le plasma, dépendent plus des variations de possibilité d'acétylation que des variations d'excrétion rénale.

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TIME-CONCENTRATION RELATIONSHIPS OF ISONIAZID WITH TUBERCLE BACILLI IN VITRO[1, 2]

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Introduction

It has been reported recently that 95 per cent of Eskimos are rapid inactivators of isoniazid, virtually no drug being present in the serum of most subjects six hours after a 4-mg. per kg. oral dose (1). In spite of this fact, the clinical impression after treating some 850 tuberculous Eskimos was that they responded as well as did other patients to the usual treatment for tuberculosis. In most cases, the treatment included a long course of isoniazid administration in doses of about 300 mg. daily.

There are at least three possible explanations for this apparent paradox: (*1*) Concentrations of isoniazid in the blood which most authorities consider too low to be maximally effective against tuberculosis in most patients are high enough to be satisfactory in the case of Eskimos; (*2*) the Eskimos were not receiving maximal benefit from the drug and could have healed their disease more quickly than they did had larger doses been given; and (*3*) some metabolic process unique to Eskimos interferes with determination of the amounts of isoniazid in the blood.

A study of serum isoniazid concentrations at different times after a 4-mg. per kg. dose showed that nearly all Eskimos, as well as all rapid inactivator non-Eskimos, had amounts of isoniazid of 0.6 γ per ml. or more at one hour, and that the great majority still had 0.4 γ per ml. or more in their serum at three hours. The question arose, therefore, whether a contact with a drug level such as, say, 0.5 γ per ml. for, say, three hours out of the day, can bring about a bacteriostatic or bactericidal effect on *M. tuberculosis*. If such a contact is ineffective in supressing growth *in vitro*, it is unlikely to be useful *in vivo*.

A search of the literature failed to elicit any information on the *in vitro* relationship between duration of daily contact of isoniazid with tubercle bacilli and the effectiveness of the drug. Some data are available on time-dose relationships in experimental animals, and deductions have also been made for humans by studying patients under treatment. But in these instances the parasite-drug effect cannot be separated from the parasite-host effect. It was thought worth while, therefore, to find out just what are the isoniazid time-concentration relationships with respect to tubercle bacilli *in vitro*. For, even taking into consideration mechanisms interfering with isoniazid action in the body, the drug-microbe reaction *in vitro* would probably be a good first approximation of the state of affairs existing within the body. It is not difficult to set up an experimental model which permits submerging tubercle bacilli in a drug-containing medium for any desired length of time and, subsequently, removing this medium, washing the bacilli, and supplying the bacteria with a drug-free medium. The procedure can be repeated daily for several days. The present paper is a detailed description of a method for making such studies and a report of the results of some preliminary tests.

Materials and Methods

In these studies, three strains of tubercle bacilli were incubated in medium containing various concentrations of isoniazid for two, three, or four hours daily for a total of six days. During the balance of each day the bacilli were incubated in plain medium. It has been found that complete or nearly complete bacteriostasis of tubercle bacilli can be brought about by 2 γ of isoniazid per ml. acting for as little as two hours daily, and a lesser but definite bacteriostatic effect can be obtained with 0.5 γ per ml. These are concentrations of isoniazid which are attainable and maintainable in the blood by many rapid inactivators, including many Eskimos. Much higher concentrations and/or longer exposures are required to bring about death of the organisms.

[1] From the Department of Laboratories of the Hamilton Health Association, Hamilton, Ontario, Canada.

[2] This investigation was supported by a Federal Health Grant.

Media Used

(*a*) *Middlebrook's 7H9 liquid medium*[3] (2–4): This consists of the basic medium described by Cohn and associates (2). Before autoclaving, to each liter of the medium are added 0.005 gm. of ferric ammonium citrate and 0.01 gm. of para-aminobenzoic acid (this last substance is incorporated only because the medium is also used routinely for diagnostic work). After autoclaving, the following components are added to each liter of the medium: 100 ml. of oleic-acid–albumin complex prepared as described by Dubos and Middlebrook (3), which has been subjected to a thirty-minute heating at 56°C.; 4 ml. of an autoclaved 50 per cent solution of dextrose in 0.005 M. of citric acid; and 3 ml. of a 1-mg. per ml. solution of catalase as described by Cohn and his co-workers (4). At this point the medium is transferred to 250-ml. Erlenmeyer flasks in 200-ml. amounts, incubated overnight to check sterility, and then refrigerated until required. On the day the medium is to be used, 5,000 units of penicillin are added to each 200-ml. amount. (It has been shown that penicillin may disappear from a similar medium under certain circumstances in a matter of days (5).)

(*b*) *Middlebrook's 7H9 liquid medium containing isoniazid:* Isoniazid is incorporated into 200-ml. lots of 7H9 liquid medium just before use from 100- and 1,000-γ per ml. solutions according to the following table:

Approximate Strength Required in the Medium (γ *per ml.*)	Strength of Isoniazid Solution to be Used (γ *per ml.*)	Amount of Solution Added (*ml.*)
16.0	1,000	3.2
8.0	1,000	1.6
4.0	1,000	0.8
2.0	100	4.0
1.0	100	2.0
0.5	100	1.0
0.25	100	0.5

It was not considered that any significant dilution of the medium occurred as a result of these additions, and no correction for this was introduced into the controls.

(*c*) *Middlebrook's 7H9 liquid medium with Tween®:* This medium is the same as (*a*) except that an albumin solution (as described by Cohn and associates (4)) replaces the albumin-oleic complex and that, on the day it is to be used, 1 ml. of a freshly made autoclaved 10 per cent solution of Tween (kept well-mixed while cooling) is incorporated in each 200 ml. The medium is tubed aseptically in 5-ml. amounts.

(*d*) *Löwenstein-Jensen medium:* The formula given by Tarshis and co-workers (6) is used. The medium is inspissated while sloped in 60-ml. bottles and is also put up in Petri dishes.

[3] With the exception of a pamphlet issued by Difco Laboratories, an entire description of Middlebrook's 7H9 liquid medium does not appear to be available in any one publication. However, Dr. M. L. Cohn kindly checked the formula employed in the present laboratory, and he recommended no changes.

Strains of Mycobacterium Tuberculosis Studied

H37Rv: Mycobacterium tuberculosis H37Rv was obtained through the kindness of Dr. William Steenken and has been maintained on Löwenstein-Jensen medium for several years.

BCG: BCG was taken from a vial of Connaught Laboratories vaccine and has been maintained for a year on Löwenstein-Jensen medium.

Strain 7373: Strain 7373 was isolated from a previously untreated patient in March, 1959.

Inocula

Six drops from a Pasteur pipette of eight-day-old cultures in 7H9 Tween medium are employed.

Description of Use of Millipore Chamber

Culture chambers: A membrane filter[4] is placed in distilled water in a Petri dish and, when thoroughly moistened, is applied to the base of a filter holder.[5] In doing this, the membrane filter is pressed out so as to be absolutely flat against the fritted glass support. The funnel of the holder is pressed in place and clamped on; then its mouth is covered with one-half of a Petri dish. This whole filtering unit is wrapped in a towel and autoclaved ten minutes at 15 pounds, using a rapid exhaust. When cold, employing aseptic technique, it is fitted firmly into the neck of a 1,000-ml. sterile filtering flask. The side tube of the latter contains an air filter of cotton wool and carries about 10 cm. of rubber tubing, the outer end of which is plugged with a glass rod. For safety, the whole apparatus is placed in a wooden holder which, in turn, is contained in a 9 × 9-inch cake pan. Leakage at the filter joint has been rare in practice, however. The top of the filter unit constitutes the chamber for the particular medium and culture in use. As long as the lumen of the rubber tubing is closed, only a negligible amount of fluid placed in the chamber passes through the filter. If, on the other hand, the lumen is patent and slight suction is applied, all fluid soon passes out of the chamber; this constitutes the procedure used for changing from one medium to another.

Culturing of test and control: Two culture chambers, one for the test and one for the control, are placed in a safety cabinet and treated as follows: The glass rods are removed from the rubber tubing of the filtering flasks. Then, proceeding with aseptic technique, a few milliliters of 7H9 medium are poured on the filters and drawn through with about 25 mm. of mercury suction to wet the filters. The rubber tubings are closed off again (from here on, clamps are used for this

[4] HA white grid-marked 47-mm. diameter filters, Millipore Filter Corporation.

[5] Millipore filter holder, Pyrex®, and rubber stopper, Millipore Filter Corporation.

purpose in preference to the glass rod), and 35 ml. of drug-containing medium warmed to 37°C. are introduced into both chambers, followed by inoculation with the strain under study.

The contents of each chamber are now stirred with a glass rod for a moment and then, in the case of the control chamber only, the medium is sucked through the filter, followed by a warm water rinse and then a rinse with plain 7H9 medium. With the tubing lumen closed off again, about 35 ml. of this medium are left in the chamber of the control. Both control and test cultures are now incubated at 37°C.

After certain intervals of time, depending on the particular experiment, the chamber with the test culture is removed from the incubator long enough to suck through the drug-containing medium and to wash it (in the same fashion as was done with the control) with warm water, then warm plain medium and, finally, to leave in place about 35 ml. of this plain medium.

On the following day, and on each of the next four days at approximately the same time of the day, the plain medium is removed from both chambers by suction, drug-containing medium is added, and the other steps are repeated as described previously.

Assessment of growth: On the seventh day, after subculturing the contents of the chambers for possible contaminants, the medium in each chamber is removed by suction. The filter holders are opened and each membrane filter is then placed aseptically on coagulated egg medium in a Petri dish (because it can be examined readily on this surface without curling up or drying, and can also be cut into strips very easily with a sterile scalpel). It is viewed with a dissecting microscope, using oblique lighting with an angle approaching horizontal, and a rough record of the number of colonies is made. If full growth has occurred, as in the controls, these stand out quite well after this period of incubation. Using aseptic technique, the filter with its culture is now divided into several short strips. One of them is placed on the surface of Löwenstein-Jensen medium in a screw-capped bottle, incubated at 37°C., and is examined at weekly intervals for eight weeks. Another is stained with neutral red by Wayne's technique (7) and examined when dry under the dissecting microscope. The others are simply kept on hand in case any special study is indicated. The number of stained colonies is recorded in terms of 0 to ++++. When any doubt arises as to whether a stained particle is a colony of tubercle bacilli or not, it is also studied with the high dry lens of an ordinary microscope after applying mounting fluid and a cover slip. With strong transmitted lighting the bacilli are easily identified.

Design of the investigation: Each of the three strains of *M. tuberculosis* was incubated for two, three, or four hours during each of six consecutive days in medium containing isoniazid, the length of contact depending on the particular experiment. The drug concentrations used ranged from 0.25 γ per ml. to 16 γ per ml. with increments in geometric progression, each succeeding strength being double the preceding one. During the balance of each day, the organisms were incubated in plain medium. The experiments were terminated on the morning of the seventh day. The amount of growth in the tests was then compared roughly with that in the controls.

Results

After six full days of incubation of the controls, a heavy growth of tubercle bacilli ranging from approximately two hundred colonies to an almost confluent mat was present on the membrane filter. As experience was gained with the technique, the growth in the controls became quite standardized, giving a colony count ranging somewhat more than two hundred. The amount of growth in the test chambers, however, was dependent upon the concentration of drug to which the bacilli had been exposed and, to a lesser extent, it also varied with the time of exposure. No concentration-time combination was used which completely prevented subsequent growth of organisms on the filter membrane when this was placed on Löwenstein-Jensen medium and further incubated. In two instances, however, with the highest concentrations and longest times, there was evidence of the production of a marked dormant, if not bactericidal, effect on most of the bacilli. Only one or two colonies grew after eight weeks' incubation following the last drug contact.

A summary of the results is presented as table 1. In this table the term "none" as applied to bacteriostasis refers to the state of affairs when growth on the test filter membrane equalled that on the control. The term "complete" signifies that no growth was present on the test, but good growth was present on the control. "Moderate" indicates approximately a quarter as many visible colonies on the test as on the control filter, while "slight" means that about three-quarters as many colonies were seen on the test filter.

It is clear from the table that bacteriostasis may be induced by exposure to fairly low isoniazid concentrations acting for as little as one-twelfth of a day, each day, for six days. Increasing the exposure time to one-sixth of a day makes very little difference in the effective concentration needed to inhibit growth. A concentration

TABLE 1

RELATIONSHIP BETWEEN THE FRACTION OF EACH DAY DURING WHICH TUBERCLE BACILLI, GROWING *in Vitro*, ARE EXPOSED TO ISONIAZID AND THE CONCENTRATION OF ISONIAZID REQUIRED TO PRODUCE A BACTERICIDAL AND/OR BACTERIOSTATIC EFFECT IN A SIX-DAY PERIOD

Isoniazid	Two Hours' Contact		Three Hours' Contact		Four Hours' Contact	
	Degree of Bacteriostatic Effect	Degree of Bactericidal Effect*	Degree of Bacteriostatic Effect	Degree of Bactericidal Effect*	Degree of Bacteriostatic Effect	Degree of Bactericidal Effect*
(γ *per ml.*)						
BCG						
16.0	complete	none	complete	none	complete	marked
8.0	complete	none	complete	none	complete	none
4.0	nearly complete	none	complete	none	complete	none
2.0	nearly complete	none	complete	none	complete	none
1.0	moderate	none	complete	none	complete	none
0.5	moderate	none	slight	none	complete	none
0.25	none	none	none	none	nearly complete	none
H37Rv						
16.0	complete	none	complete	none	complete	none
8.0	complete	none	complete	none	complete	none
4.0	complete	none	complete	none	complete	none
2.0	complete	none	complete	none	complete	none
1.0	moderate	none	complete	none	complete	none
0.5	moderate	none	slight	none	slight	none
0.25	slight	none	none	none	none	none
Wild strain 7373						
16.0	complete	none	complete	none	complete	marked
8.0	complete	none	complete	none	complete	none
4.0	complete	none	complete	none	complete	none
2.0	complete	none	complete	none	complete	none
1.0	complete	none	complete	none	complete	none
0.5	slight	none	moderate	none	complete	none
0.25	none	none	slight	none	complete	none

* As determined by a rough colony count after incubating the membrane filter for eight weeks on Löwenstein-Jensen medium.

of 16 γ per ml. for four hours seems to be capable of killing some BCG and strain 7373 organisms.

DISCUSSION

It is believed that the method employed in this investigation for determining the time-concentration relationships of isoniazid with tubercle bacilli *in vitro* is reliable so far as the detection and approximate measurement of the degree of bacteriostasis are concerned. As a precaution, the controls were subjected to a short daily drug exposure to compensate for any possible adsorption of isoniazid on the part of the tests. This phenomenon might otherwise have been suspected of playing a part in inhibiting growth of the test strains during that part of each day during which they were incubated with plain medium. But this was not the case. Moreover, a separate experiment was done in which some medium, containing drug in high concentrations, was allowed to trickle through a filter for several days, after which treatment the filter was rinsed, inoculated with bacilli, and incubated on solid medium. A normal growth of organisms occurred on the membrane under these conditions, showing that the filter did not retain any significant amount of drug even after prolonged contact with it. In the main experiments, the washing technique for test and control was identical; therefore, the only significant difference between the two was that the control organisms had only

a few minutes of daily contact with isoniazid while the tests had a long exposure.

Whereas the controls employed were entirely satisfactory for the detection and rough measurement of the degree of bacteriostasis, and also for detecting a complete or nearly complete killing effect on the bacilli, they were not adequate for assessing the proportion of organisms destroyed when the "killing" effect was small. For this purpose, a second type of control would have had to be set up. The reason for this is that colonial development in the liquid medium is accompanied by some dispersed growth. Consequently, on placing the control filter membranes on solid medium at the end of the six-day period, in addition to continuing the culture of colonies already visible microscopically, new colonies from the dispersed clumps of bacilli were also being grown. A very heavy, almost confluent, growth resulted in about one week. Nevertheless, evidence was produced that the bacilli were either rendered dormant for a long time or even actually killed when 16 γ per ml. were in contact with BCG and strain 7373 for four hours a day for six days.

A six-day experimental period was selected because good growth of control cultures is easily visible under the low power of the dissecting microscope in that length of time. It is probable that in future experiments, if the number of days is increased, lower drug concentrations and shorter exposures will be shown to be effective. The possibility and practicability of extending the duration of the tests is being explored. It is believed that the membrane filters will last for some time longer without becoming clogged or disrupted.

Supplementary experiments are now being carried out in order to throw light on the time-concentration relationship over the whole twenty-four-hour period. Finally, other strains of *M. tuberculosis* and other mycobacteria are being studied. In the meantime, it was thought that the information so far obtained might be of interest to others.

All that can be said at the present time is that, in the case of BCG, H37Rv, and a wild strain of tubercle bacilli (designated 7373) from an untreated patient, isoniazid does not have to be present any longer than two hours a day in a concentration of 2 γ per ml. for six consecutive days to accomplish a marked bacteriostatic effect, while even 0.5 γ per ml. produces some inhibition of growth.

Summary

A method is described for determining the influence of isoniazid upon three different strains of tubercle bacilli when they are incubated in the presence of various concentrations of the drug for only two, three, or four hours of each of six consecutive days, incubation being continued for the balance of each day in plain medium. Complete or nearly complete bacteriostasis is accomplished by a contact with isoniazid concentrations as low as 2 γ per ml. for two hours, and some effect is produced by 0.5 γ per ml. for the same length of time. Much higher concentrations and longer exposures are required to bring about a bactericidal change.

Sumario

Las Relaciones en Tiempo-Concentración de la Isoniacida con los Bacilos Tuberculosos in Vitro

Descríbese un método para determinar el influjo de la isoniacida sobre tres distintas cepas de bacilos tuberculosos cuando se incuban éstas en presencia de varias concentraciones de la droga solamente durante dos, tres o cuatro horas de cada uno de seis días consecutivos, continuándose la incubación durante el resto de cada día en medio simple. Se logra bacteriostasia total o cuasitotal por el contacto con concentraciones de isoniacida que no pasan de 2 γ por ml. durante dos horas y se produce algún efecto con 0.5 γ por ml. durante el mismo período de tiempo. Para producir una alteración bactericida se necesitan concentraciones mucho más altas y exposiciones más largas.

Resume

Rapports temps-concentration de l'isoniazide avec les bacilles tuberculeux in vitro

L'auteur décrit une méthode de détermination de l'influence de l'isoniazide sur trois différentes souches de bacilles tuberculeux, mises à incuber avec des concentrations différentes du médicament, pendant seulement deux, trois ou quatre heures par jour, pendant six jours consécutifs, l'incubation étant continuée le reste de la journée dans un milieu ordinaire. Une destruction complète ou presque complète des bacilles est accomplie par un contact avec l'isoniazide à une concentration aussi faible que 2 γ par ml, pendant deux heures et il y eut quelqu'effet à la concen-

tration de 0,5 γ par ml, pendant la même durée de temps. Des concentrations plus fortes et des expositions plus longues sont nécessaires pour obtenir un changement bactéricide.

Acknowledgments

Considerable credit for the development of the fine details of the culturing technique is due Miss Florence M. Gray and Miss Eleanor D. Foster.

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RELATIVE HUMIDITY AND AIR-BORNE INFECTIONS[1]

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Introduction

Possible relations between the weather and health have been suggested frequently. In the present discussion the term "weather" will be restricted to relatively short-time local variations in meteorologic phenomena, as distinct from seasons and climates. The special topic will be the presentation of a possible relationship between relative humidity and the onset of the 1957 influenza epidemic in the United States.

Observations

Some Biologic Effects Dependent on Relative Humidity

In a recent discussion, Williams (1) has classified the possible effects of weather on humans as follows: general systemic, local respiratory, microbial, and effects on group behavior. The present discussion will be concentrated on microbial effects while recognizing, however, the importance of group behavior as related to the heating season, the school term, and the predominance of concentration in groups during the daytime. For an air-borne respiratory infection, such as influenza, passage through the atmosphere is an essential part of the infective chain and, therefore, the microbial agents will be exposed to the effects of relative humidity.

The influenza virus leaves an infected person via a sneezed droplet in which the virus will be suspended in saliva. Saliva contains sodium chloride (of the order of 0.5 mg. per ml.) (2) as well as other biologically important materials, including a probable inhibitor with which the virus may be associated. The sneezed droplet leaves an environment at 100 per cent relative humidity and enters the ambient atmosphere, which is usually at some lower relative humidity. The droplet will therefore lose water by evaporation until further decrease in size is limited by reduction in vapor pressure due to the increased concentration of the dissolved chemicals; or the evaporation may proceed to the limit at which the solutes crystallize. This evaporation process will be followed through in some detail since it may have a decisive influence on the ability of the expelled virus to cause infection.

The distribution of size of sneezed droplets has been investigated by Jennison (3) who found that 30 per cent of sneezed droplets have a radius of less than 25×10^{-4} cm. about 0.2 seconds after the start of a typical sneeze. Since the smaller droplets, which will remain air-borne, are of greatest interest, a droplet of initial radius 17×10^{-4} cm. and containing 10^{-11} gm. of sodium chloride (NaCl) will be selected as the typical droplet. The droplet will contain other chemicals, probably including an inhibitor. The influenza virus has a radius of about 5×10^{-6} cm. so that it can be accommodated comfortably within the typical droplet.

The evaporation behavior of the typical droplet when sneezed into an atmosphere of given relative humidity may be estimated by extrapolation from data quoted by Mason (4) for droplets containing various amounts of sodium chloride. These data for equilibrium radii are shown in figure 1 together with data on the rates of fall of particles of different radii. Certain properties of droplets containing 10^{-11} gm. of sodium chloride which have reached equilibrium in various ranges of relative humidity (RH) are listed in table 1.

These four ranges of ambient humidity have been selected because it is to be expected that certain important effects, to be discussed subsequently, will be of predominant importance in each of these ranges. It should be emphasized, however, that the distinction between these ranges is obviously not as sharp as might be inferred from the table.

The important characteristics of range 1 (100–96 per cent) are that the droplets remain relatively large and the concentration of sodium chloride is relatively low. The droplets will therefore tend to fall out of suspension and there will also be little opportunity for abnormal chemical interaction between the solute and the virus.

In range 2 (95–86 per cent), the smaller size will keep the droplets air-borne longer, and

[1] From the General Electric Research Laboratory, Schenectady, New York.

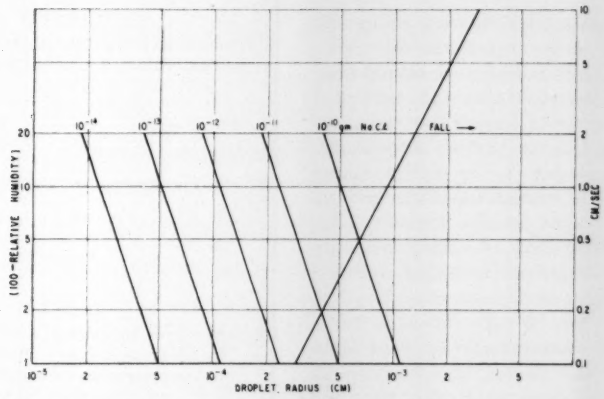

FIG. 1. Physical properties of water droplets containing various amounts of sodium chloride when injected into atmospheres of various relative humidities:
Left: Equilibrium droplet radius vs. (100 − relative humidity)
Right: Equilibrium droplet radius vs. rate of fall

therefore give increased opportunity for them to be inhaled. A possibly more important effect is that, if the virus is associated with an inhibitor, the salt concentration may be high enough in this range to dissociate virus from inhibitor. It has been shown (5) that antibody may be separated from pneumococci by treatment with a solution containing 150 mg. of sodium chloride per ml. The droplet concentrations in range 2 are from 98 to 250 mg. per ml. It is not known whether this particular chemical treatment is effective in separating influenza A virus from the inhibitor, but other chemical treatments have been shown to be effective. Separation of virus from inhibitor would, presumably, greatly increase the chances of infection if the droplet were inhaled.

In range 3 (85–76 per cent), it is to be expected that the sodium chloride concentration will be great enough to kill the virus in the air-borne droplet. Dunklin and Puck (6) and Lester (7) have shown that, if droplets of sodium chloride solution, containing bacteria or influenza virus, respectively, are sprayed into an atmosphere of intermediate relative humidity, the microorganisms are killed promptly by the salt concentrations which build up as the droplets evaporate to equilibrium radius. The presence of sodium chloride was essential to killing. Lester's zone of killing extended from 30 per cent relative humidity to 80 per cent relative humidity. Since longer exposures are involved in practice than in Lester's experiments (many virus droplets will remain air-borne for long periods at these humidities) the upper limit for killing will be increased to 85 per cent relative humidity. The lower relative humidity limit for killing depends on the crystallization behavior of the saturated salt solution. If adequate nuclei for crystallization are present, the solution will crystallize at 76 per cent relative humidity or a little higher (normal saturation 360 mg. per ml.). In Lester's experiments the droplets were very small (50 per cent of the mass in droplets of radius $<1.5 \times 10^{-4}$ cm.), so that the chance of finding a nucleus in a given drop was small, and rapid crystallization would probably occur only at very strong supersaturation. Lester therefore observed his maximal killing at lower humidities such as 50 per cent, so

TABLE 1
PROPERTIES OF TYPICAL DROPLET WHICH REACHED EQUILIBRIUM IN VARIOUS RANGES OF RELATIVE HUMIDITY

	Relative Humidity Range	Radius	Rate of Fall	NaCl Concentration	Stability in Atmosphere
	per cent	10^{-4} *cm.*	*cm./sec.*	*mg./ml.*	
0	Initial	17	3.5	0.5	—
1	100–96	17–3.2	3.5–0.12	0.5–73	Fall out
2	95–86	2.9–2.1	0.10–0.054	98–250	Air-borne
3	85–76	2.0–1.7	0.049–0.035	295–480	Air-borne
4	<75	1.0	0.012	Crystal	Air-borne

that the virus was exposed to very strong salt solutions as the drop evaporated to smaller radii. The typical saliva droplet considered here, which is initially some 1,500 times the volume of Lester's droplets, would have 1,500 times the chance of containing a nucleus and would, therefore, be expected to crystallize rather promptly from a somewhat supersaturated solution at 76 per cent relative humidity. Accordingly, the killing range of relative humidity will be set at 76 to 85 per cent instead of extending into Lester's range of lower humidities.

In range 4 (below 76 per cent) it is to be expected that the abundant nuclei present in the typical drop in the present study will cause crystallization to occur so rapidly that there will be too little time for the virus either to be separated from the inhibitor or to be killed by strong salt solutions. The live virus (perhaps associated with the inhibitor) will remain attached to the salt crystal (10^{-4} cm. radius) and suspended in the atmosphere. Lester has shown that live virus can be recovered from a rapidly crystallized droplet.

The spread in sizes of the droplets in ranges 2, 3, and 4 above means that, when inhaled, they will tend to be deposited in different parts of the respiratory tract, the smallest particles penetrating farthest. This is illustrated by data (8) on the probability of deposition of inhaled particles in the upper respiratory tract shown in table 2.

The pathology of influenza appears in the upper respiratory tract, but it has not been demonstrated conclusively that this is also the primary seat of infection. Accordingly, although the sizes and consequent places of deposition may be expected to influence the infectivity of inhaled droplets containing virus, it cannot be said categorically that the droplets of range 2 (95–86 per cent) will be more infective than those of range 4 (<76 per cent) simply because their larger size will cause them to be deposited higher up in the respiratory tract. However, this possibility, together with the possibility that the virus in range 2 will be dissociated from the inhibitor, while the virus in range 4 is more likely to be associated with the inhibitor, makes it reasonable to explore the consequences of assuming greater infectivity for the droplets of range 2 than for those of range 4 (table 3).

Since in many cases the humidity cycles daily through the 76–85 per cent killing range, it is likely that virus associated with salt crystals and stored in the air or on surfaces will not survive long before the humidity rises into the killing range. It is only in exceptional cases, therefore, that there may be effects due to virus stored on surfaces and later liberated into the air. Such effects may be prominent indoors during the heating season and in places of abnormally low relative humidity, such as Salt Lake City.

TABLE 2

PROBABILITY OF DISPOSITION OF INHALED PARTICLES IN UPPER RESPIRATORY TRACT

Radius	Per Cent Deposited in Upper Respiratory Tract
0.6×10^{-4} cm.	0
1.0	33
1.5	56
2.5	73

TABLE 3

SUMMARY OF BEHAVIOR OF SALIVA DROPLET CONTAINING VIRUS SNEEZED INTO ATMOSPHERES IN VARIOUS RANGES OF RELATIVE HUMIDITY

Range	Biochemistry in Atmosphere	Effects from Inhalation
1	None	Not inhaled
2	Possible dissociation of virus from inhibitor by NaCl solution of moderate concentration	(a) Droplet deposited preferentially in upper respiratory tract (b) Virus alive and possibly dissociated from inhibitor
3	Virus killed by NaCl solution of high concentration	None: virus dead
4	Virus alive, attached to salt crystal, and possibly associated with inhibitor	(a) Droplet deposited preferentially in lower respiratory tract (b) Virus alive, and possibly associated with inhibitor

Characteristics of an Epidemic

Before attempting to apply the weather criterion implicit in table 3 to the onset of the 1957 influenza epidemic, it will be worth while to review briefly some of the characteristics of an epidemic.

There will be an epidemic only when an infected person on the average infects more than one sus-

TABLE 4

RELATION BETWEEN TOTAL FRACTION OF SUSCEPTIBLES INFECTED IN COURSE OF EPIDEMIC AND MULTIPLICATION CONSTANT

Total Fraction of Susceptibles Infected	Multiplication Constant
0.00	1.000
0.10	1.054
0.20	1.116
0.30	1.189
0.40	1.277
0.50	1.386
0.60	1.527
0.70	1.720

ceptible person. Or, by analogy with thermal nuclear reactors which are now so familiar, there will be no chain reaction unless the multiplication constant exceeds unity.

Most of the requirements for an epidemic may be present: a source, susceptibles, crowd habits, schools, et cetera; but there will be no epidemic unless the essential component for transfer of the virus, favorable weather, is also present. In the thermal reactor analogy, source and fissionable material may be present in abundance, but there will be no chain reaction unless a moderator is also present to modify the neutrons produced by fissioning atoms so that they can cause the fission of other atoms.

Even after the favorable weather occurs there will be a delay before the epidemic can build up to recognizable size, just as there is a delay in reaching substantial power after a reactor "goes critical." For the influenza epidemic this delay has two causes. The first is that in a city there are always some cases of respiratory illness, so that an additional cause of respiratory illness must affect about 1 per cent of the population before it becomes noticeable. This means that for a city of 100,000 the original single source of infection must have spread to a thousand people before it will be recognized. The second cause is related to the incubation period of the disease and to the fact that the multiplication constant is usually not much greater than unity, so that each infected person infects on the average not much more than one susceptible. This has been shown in the theory of epidemics by D. G. Kendall (9) who has derived on quite general assumptions a relation between the total fraction of susceptibles who become infected during the course of the epidemic and the multiplication constant, as shown in table 4.

Thus, it is possible to have a very severe epidemic when the multiplication constant is 1.5. The incubation period for influenza is about two days so that, under the conditions above, about fifteen days would be required for an initial single infection to spread to 1,000 people. It is therefore to be expected that, if weather conditions are important, a period of about fifteen days will have elapsed between the occurrence of unusually favorable humidity conditions in the presence of a source and the recognizable appearance of the epidemic.

Most of the United States was seeded with Asian influenza by June, 1957, but an epidemic did not appear in most places until October, 1957. The attempt will be made to connect this delayed onset with humidity conditions. It is thought that the determination of onset time is a simpler problem than the study of severity, since the latter depends on several factors—other than weather—which may vary substantially from city to city.

Available Data on the 1957 Influenza Epidemic

Data on employee absenteeism in 36 cities of the United States have been published by the U.S. Public Health Service (10). These data are probably the best available measure of the onset and severity of the epidemic (11). Conditions for spreading the epidemic will be most favorable when people are congregated in schools, places of business, and so on. This places two important restrictions on the meteorologic data to be used. First, consideration will be limited to relative humidity data between the hours of 0800 and 2000, due allowance being made for Daylight Saving Time. Second, the humidity data from meteorologic records are taken out-of-doors and, therefore, consideration should be limited to those cities which had sufficiently warm weather throughout the epidemic onset period to make the relative humidity data approximately valid indoors where people are congregated.

Of the warmer cities, Jacksonville, Los Angeles, and San Francisco had very little employee absenteeism. Houston, Dallas, and Miami had substantial employee absenteeism and also had sufficiently warm weather. Nashville, Pittsburgh, and Philadelphia were three cities in which the onset of the epidemic was particularly well marked, but the weather was rather cold in November. Consideration will be limited therefore to these six last-named cities.

In table 5 the data are given for employee absenteeism (per cent EA), hours in relative

TABLE 5

DATA ON EMPLOYEE ABSENTEEISM AND HOURS IN VARIOUS RANGES OF RELATIVE HUMIDITY IN SIX CITIES FROM JULY THROUGH DECEMBER, 1957

	Maximal November Temperature	July, 1957			August, 1957			September, 1957		
		EA*	76–85	86–95	EA	76–85	86–95	EA	76–85	86–95
Houston	69.6	—	44	11	—	56	17	—	55	54
Dallas	60.8	—	10	0	—	10	3	—	33	14
Miami	79.3	—	79	38	—	111	48	—	131	38
Nashville	58.2	—	46	23	—	22	16	—	78	76
Philadelphia	55.9	—	30	12	—	37	18	—	66	47
Pittsburgh	50.8	—	43	27	—	33	27	—	50	66
		October, 1957			November, 1957			December, 1957		
Houston	69.6	5.4	54	53	4.3	73	105	4.0	65	72
Dallas	60.8	6.4	32	43	7.4	66	50	5.1	35	50
Miami	79.3	10.3	75	36	7.6	89	55	7.9	93	55
Nashville	58.2	6.2	52	73	7.0	60	55	4.3	58	72
Philadelphia	55.9	8.9	34	46	6.3	47	51	5.9	49	72
Pittsburgh	50.8	9.4	68	72	5.2	75	50	5.0	83	81

* Percentage of employee absenteeism. Dash indicates normal absenteeism.

humidity ranges 2 (95–86 per cent) and 3 (85–76 per cent) for the period between 0800 and 2000 hours, for the months July through December, 1957, together with maximal November temperatures. A dash indicates that the employee absenteeism was reported as normal in all cities for July through September, 1957. Corresponding data are given in table 6 on absenteeism for October through December, 1956. This was a "normal" year, and the sharp increase in absenteeism in October 1957 over 1956 for all the cities except Miami (*see* below) is evident. Absenteeism data are available (10) for part of 1958, but have not been quoted in table 5 as the concern here is only with the onset—in October in most cases. It will be evident from table 5 that, of the 390 hours per month lying between 0800 and 2000, from 10 to 178 hours per month lie in humidity ranges 2 (86–95 per cent) and 3 (76–85 per cent). Most of the hours lie at lower humidities.

TABLE 6

DATA ON EMPLOYEE ABSENTEEISM IN SIX CITIES FROM OCTOBER THROUGH DECEMBER, 1956

	October, 1956	November, 1956	December, 1956
Houston	4.0	4.1	3.4
Dallas	4.3	3.8	4.4
Miami	10.3	7.3	7.4
Nashville	3.6	3.4	4.1
Philadelphia	5.3	5.3	5.8
Pittsburgh	4.5	3.7	4.3

Comparison of Relative Humidity Data with Onset of Epidemic

A general view of a fairly typical relative humidity pattern throughout one year is given in figure 2, which is a bar chart for Houston, showing by months from July, 1957, through June, 1958, the number of hours between 0800 and 2000 spent in various 5 per cent intervals of relative humidity. These eighteen intervals range from 96–100 per cent at the right of each month to 11–15 per cent at the left. It is evident that during the summer there is a strong negative slope in going from low to high humidities at the high end of the abscissa scale, whereas in the autumn (*see* October, November) this trend is decreased or reversed.

This fact, together with the preceding discussion of physical and biochemical effects in various ranges of humidity, suggests that a match between humidity conditions and employee absenteeism might be obtained by observing the changes by months of the ratio

$$\frac{\text{number of hours at 86\% to 95\% RH between 0800 and 2000}}{\text{number of hours at 76\% to 85\% RH between 0800 and 2000}}$$

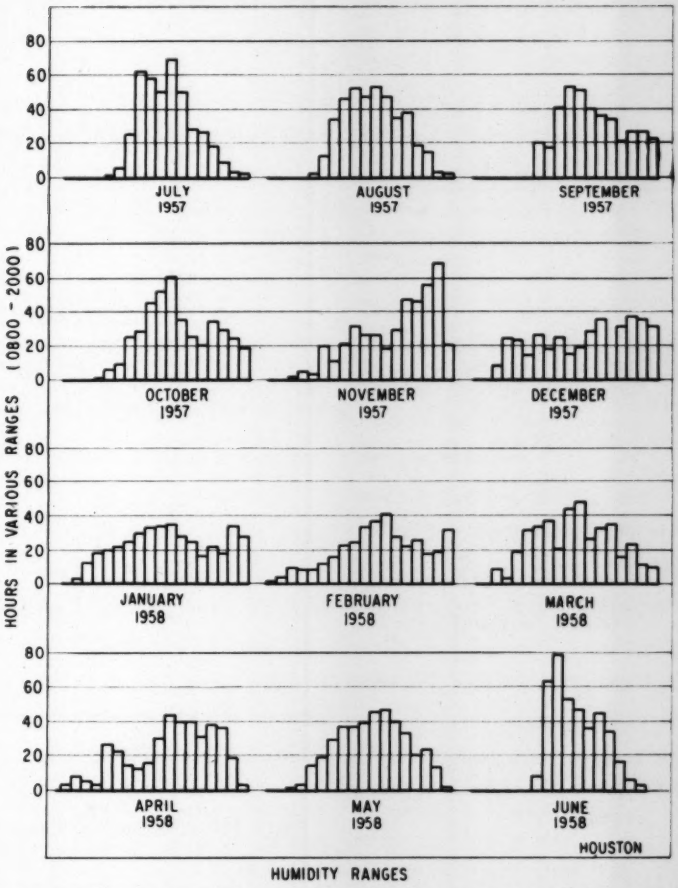

FIG. 2. Relative humidity data for Houston, Texas, July, 1957–June, 1958. The ordinates show for each month the number of hours between 0800 and 2000 spent in various 5 per cent intervals of relative humidity, ranging from 11–15 per cent to 96–100 per cent (the highest range is on the right of the abscissa scale).

This ratio compares the number of hours favorable to infection by inhaled virus with the number of hours in which air-borne virus will be killed by relative humidity effects. The pattern of figure 2 shows that this ratio will increase sharply in the autumn and therefore would match the sudden onset at that time of absenteeism in a community which had been seeded with influenza since June, 1957.

In figure 3 are shown plots of the above humidity ratio, and of the 1957/1956 ratio of absenteeism for Houston, by months for the period July, 1957 to April, 1958. These plots show a relationship of the expected kind, that is, the humidity ratio is low in July and August, rising to approximately unity in September, and this rise occurs before the rise in the absenteeism ratio which takes place in October. The humidity ratio also remains at high values during the period of substantial abnormal absenteeism. Detailed agreement between the shapes of the two plots cannot be expected since the intensity and duration of the epidemic involve several factors besides the humidity ratio. That is, severity is a more complicated matter than onset. In this and all other figures, all of the available

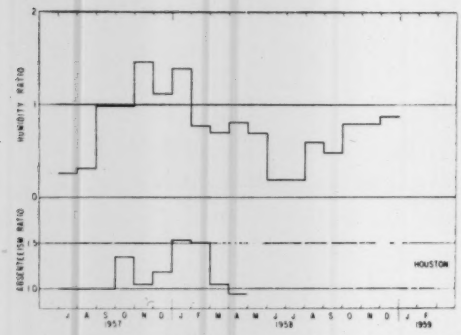

FIG. 3. "Humidity ratio" and "absenteeism ratio" data for Houston, Texas, as a function of months for July, 1957, through 1958. The humidity ratio is defined as:

$$\frac{\text{number of hours at 86 to 95\% RH between 0800 and 2000}}{\text{number of hours at 76 to 85\% RH between 0800 and 2000}}$$

The absenteeism ratio is defined as:

$$\frac{\text{employee absenteeism during 1957–1958 influenza epidemic}}{\text{employee absenteeism during 1956–1957 corresponding period}}$$

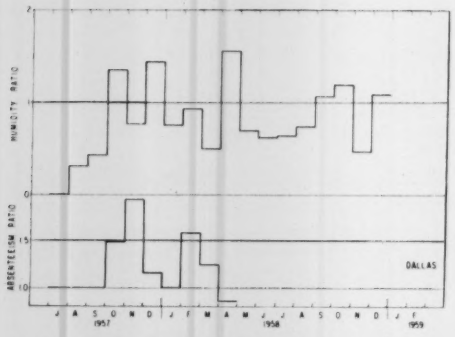

FIG. 4. Humidity ratio and absenteeism ratio data for Dallas, Texas. (*See* figure 3 for definitions.)

employee absenteeism data are shown, and the absenteeism ratio was said to have been normal (unity) through July, August, and September, 1957.

Similar data for Dallas are shown in figure 4, and again there is some correlation between the onset of abnormal employee absenteeism and the appearance of large values of the humidity ratio.

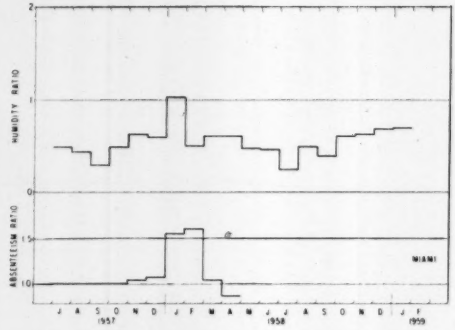

FIG. 5. Humidity ratio and absenteeism ratio data for Miami, Florida. (*See* figure 3 for definitions.)

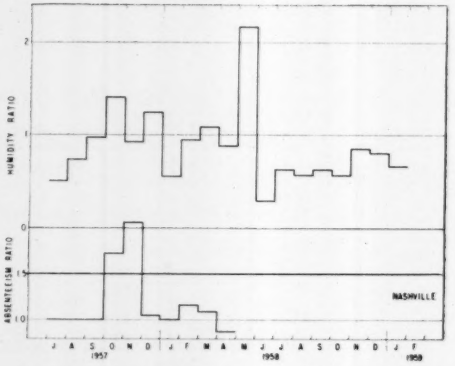

FIG. 6. Humidity ratio and absenteeism ratio data for Nashville, Tennessee. (*See* figure 3 for definitions.)

Similar data for Miami may be seen in figure 5. This city is unique in that the onset of abnormal absenteeism was delayed until January, 1958, that is, until long after the opening of school in September. The humidity ratio plot also shows this delay quite satisfactorily in that there was an increase to a large value in January, 1958.

Similar data for Nashville, Philadelphia, and Pittsburgh are shown in figures 6, 7, and 8, respectively. Relatively humidity ratios are shown throughout the entire period July, 1957, to January, 1959, although, as noted above, these ratios can have little significance indoors during the heating season. All three figures show a correlation between the occurrence of large relative humidity ratios and the onset of absentee-

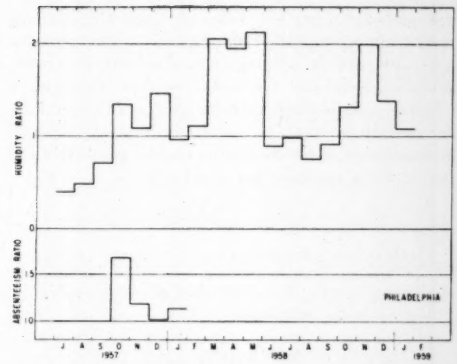

FIG. 7. Humidity ratio and absenteeism ratio data for Philadelphia, Pennsylvania. (*See* figure 3 for definitions.)

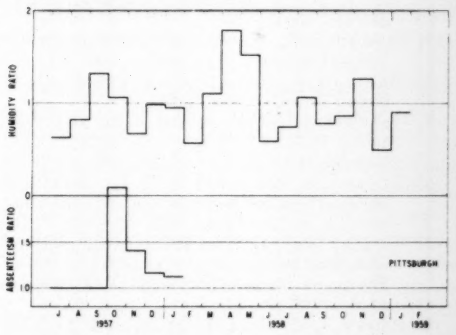

FIG. 8. Humidity ratio and absenteeism ratio data for Pittsburgh, Pennsylvania. (*See* figure 3 for definitions.)

ism. It may be significant that the relative humidity ratio is highest in September in Pittsburgh (1.32), and Pittsburgh was the city among these three which experienced the most rapid and severe onset of absenteeism in October.

A comparison of relative humidity conditions for an influenza year and a normal year is shown in table 7, in which the humidity ratios are listed for the six cities for the critical months of September through January (Miami) for the 1957–1958 and 1958–1959 seasons. It may be seen in the table that for all cities except Philadelphia the humidity ratio was more favorable for epidemic conditions in 1957–1958. Employee absenteeism data for 1958–1959 are not available at present.

COMMENT

It is unfortunate that the amount of data is so limited for testing the hypothesis of a correlation between relative humidity conditions and the onset of the 1957 influenza epidemic. Something might be done with the colder cities by assuming identical *absolute* humidity indoors and outdoors, and calculating the *relative* humidity indoors by assuming a constant temperature there of 70°F. The validity of this assumption has not been explored, and the data have not been treated in this way as yet. Perhaps the major point of the present study is to suggest the importance of looking for relations between humidity conditions and epidemics.

SUMMARY

The possible role of relative humidity in the spread of air-borne infection is discussed. Details are given for the evaporation of water from droplets of saliva containing virus which are sneezed into atmospheres of various relative humidities. The resulting biochemical interactions of the virus (and possibly the associated inhibitor) with the concentrated sodium chloride solutions produced in the droplets are explained. It is shown that the relative humidity conditions

TABLE 7

COMPARISON OF HUMIDITY CONDITIONS FOR A NORMAL YEAR AND AN "INFLUENZA" YEAR (SEPTEMBER THROUGH JANUARY, 1957–1958 AND 1958–1959)

	1957–1958					1958–1959				
	S	O	N	D	J	S	O	N	D	J
Houston	0.98	0.98	1.44	1.11	1.37	0.47	0.79	0.79	1.07	—
Dallas	0.42	1.34	0.76	1.43	0.75	1.06	1.18	0.46	1.08	—
Miami	0.29	0.48	0.62	0.59	1.03	0.38	0.60	0.62	0.68	0.69
Nashville	0.97	1.40	0.92	1.24	0.55	0.62	0.56	0.85	0.80	0.66
Philadelphia	0.71	1.35	1.09	1.47	0.96	0.91	1.32	2.00	1.39	1.08
Pittsburgh	1.32	1.06	0.67	0.98	0.95	0.78	0.86	1.26	0.49	0.90

usually are unfavorable for the transmission of air-borne infection. It is suggested that a particular pattern of relative humidity values will favor the transmission of air-borne infection. This criterion is applied to the onset of the 1957 influenza epidemic in a number of cities in the United States for which data on employee absenteeism are available.

Sumario

La Humedad Relativa y las Infecciones Aeróferas

Se discute el posible papel de la humedad relativa en la propagación de la infección transportada por el aire. Se ofrecen pormenores relativos a la evaporación de agua procedente de las gotillas de saliva que contiene virus cuando se estornudan en atmósferas de varias humedades relativas. Se explican las resultantes interacciones bioquímicas del virus (y posiblemente del inhibidor asociado) con soluciones concentradas de cloruro de sodio. Demuéstrase que las condiciones de humedad relativa suelen ser desfavorables para la transmisión de infección aerófera. Se sugiere que una forma dada de valores de humedad relativa fomentará la transmisión de dicha infección. Se aplica esta pauta a la iniciación de la epidemia de influenza de 1957 en varias ciudades de los Estados Unidos para las cuales se cuenta con datos referentes al absentismo de los empleados.

Resume

Humidité relative et infections transmises par air

L'auteur discute le rôle possible de l'humidité relative dans la propagation par air des infections. Il donne des détails sur l'évaporation de l'eau de gouttelettes de salive contenant des virus et émises par éternuement dans l'atmosphère à différentes humidités relatives. Il explique les interréactions biochimiques qui en résultent pour les virus (et peut-être pour l'inhibiteur associé) mis en contact avec des solutions concentrées de chlorure de sodium. Il est démontré que des conditions d'humidité relative sont habituellement défavorables à la propagation des maladies transmissibles par air. L'auteur émet l'hypothèse qu'un type particulier de degré d'humidité relative favorise la propagation d'infections transmissibles par air. Ce critérium est appliqué à l'apparition de l'épidémie de grippe de 1957, dans un certain nombre de villes des Etats-Unis dans lesquelles on a pu obtenir des renseignements sur les jours d'absence des employés.

Acknowledgment

Grateful acknowledgment is made to G. J. Hahn for assistance in the initial collection of data, and to Miss Carolyn Weschrob for help with the processing of the data.

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THE VALIDITY OF SOCIO-ECONOMIC DIFFERENTIALS IN TUBERCULOSIS MORTALITY[1]

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INTRODUCTION

The inverse relationship of socio-economic status and tuberculosis mortality has been demonstrated in numerous studies (1–5). Moreover, the data indicate that tuberculosis is undoubtedly one of the diseases most sensitive to socio-economic differentials, and it has been generally concluded that factors associated with low socio-economic status play an important causative role in tuberculosis mortality.

Such studies have been criticized, however, on the grounds that the disease itself might be the cause of low socio-economic status. For example, Terris (6), studying tuberculosis deaths in Buffalo in the years 1939–1941, found the inverse correlation of economic status and tuberculosis mortality to be greater for adult males than for adult females. He concluded that economic status per se is not the only factor involved and that other associated factors, more or less limited to males, were responsible for the large differences found. Lilienfeld and Korns (7), using the same methods, obtained similar results for cirrhosis of the liver. They pointed out, however—and the criticism applies to the study by Terris as well as their own—that "It is quite possible that a chronic illness, such as cirrhosis, has been the cause, rather than the result, of the decreased economic status and other associated factors among the males. To the writers, this explanation seems less probable but cannot be dismissed on the basis of the available data."

Similarly, Anderson, Enterline, and Turner (8) refer to various studies demonstrating the inverse relationship between tuberculosis mortality and economic status, and make the comment that "Because tuberculosis is likely to cause considerable economic distress during its course, the economic status of patients is likely to change as the disease progresses." Sartwell and Merrell (9), in a discussion of chronic disease morbidity rates, have pointed out that "If the prevalence of a disabling chronic disease is found to vary inversely with economic level, the explanation may either be that environmental influences associated with poverty predispose to the disease or that the existence of the disease has forced the individual or family downward into a lower stratum."

The present study was undertaken in an attempt to determine whether such downward "drift" or change in socio-economic status prior to death is responsible for the inverse relationship between tuberculosis mortality and socio-economic status. It is believed that the issue in question represents a significant methodologic problem in epidemiologic studies of chronic disease, and that study of a particular chronic disease—tuberculosis—might be helpful in providing some of the data necessary to resolve the problem.

METHODS

The methods used were similar to those employed in a previous study of schizophrenia (10). The names of all Buffalo residents reported to have died from tuberculosis during the three-year period 1949–1951 were obtained from the New York State Department of Health. For each person, further data were obtained from the Bureau of Biostatistics and the Chest Clinic of the Erie County Health Department; these included address at death, age, and sex, as well as information on marital status and names of relatives to serve as aids in tracing addresses. Previous addresses were traced through the use of the Buffalo city directories and records of admissions to the E. J. Meyer Memorial Hospital in Buffalo and the J. N. Adam Memorial Hospital in Perrysburg, New York.

The city's census tracts were grouped into four economic quartiles of approximately 150,000 population each on the basis of median monthly rentals as reported in the 1950 census. Since census tracts were first set up in 1930, Buffalo addresses were traced back no farther than 1925. The economic quartiles used for addresses that were traced back to the 1925–1934 and 1935–1944 periods were those obtained by grouping census tracts according to median monthly rentals reported in the 1930 and 1940 censuses. However, there was actually very little change in the census tract groupings during this twenty-year period.

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TABLE 1

ADJUSTED TUBERCULOSIS MORTALITY RATES OF WHITE POPULATION BY SEX, MAJOR AGE GROUPS, AND ECONOMIC QUARTILE, BUFFALO, 1949–1951

Economic Quartile at Death	Adjusted Mortality Rates		
	Less than 35 Years	35 Years and Older	Total
Males			
I (highest)	5.3	33.4	18.6
II	10.1	53.7	30.7
III	7.9	67.4	35.9
IV (lowest)	16.1	212.5	108.6
Females			
I (highest)	4.8	10.4	7.4
II	9.5	16.8	12.9
III	18.0	11.0	14.7
IV (lowest)	13.5	31.0	21.8

TABLE 2

NUMBER AND PER CENT, BY SEX, OF DEATHS FROM TUBERCULOSIS OF WHITE PERSONS, BUFFALO, 1949–1951, TRACED TO DIFFERENT TIME PERIODS

Time Period Traced to	Males		Females		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
1947–1951	69	18.2	12	10.6	81	16.4
1936–1946	67	17.6	21	18.6	88	17.9
1925–1935	244	64.2	80	70.8	324	65.7
Total	380	100.0	113	100.0	493	100.0

RESULTS

Tuberculosis mortality rates by age and sex were calculated for the white population in each of the economic quartiles. Similar comparisons for the nonwhite population were not made because the nonwhite population in the two highest quartiles is negligible. The results are shown in table 1.

It will be noted that, for both males and females less than thirty-five years of age and for females thirty-five years and older, the mortality rate in the lowest economic quartile is about three times that in the highest economic quartile. For males thirty-five years and older, the mortality rate in the lowest economic quartile is more than six times that in the highest economic quartile. These differences are similar to but greater than those found in the previous study of 1939–1941 deaths (6).

The success in tracing the cases to early residences in Buffalo appears in table 2. Eighty-four per cent were traced back to 1946 or earlier, and 66 per cent were traced back to 1935 or earlier.

The results of the tracing are shown in table 3. It will be noted that the distribution of tuberculosis cases among the economic quartiles is not significantly different at an earlier date from that at time of death.

It should also be noted that 42 of the 208 males in quartile IV could not be traced. Comparison of these 42 males with the 166 who were traced showed them to include a significantly higher proportion of single individuals and of persons born in states other than New York. No significant differences were found in age distribution or in types of occupation: both groups consisted predominantly of unskilled and semiskilled workers, approximately 15 per cent were skilled workers, and 5 per cent were white collar workers, professionals, and proprietors.

An additional analysis was made for those persons who presumably had the greatest opportunity to change economic quartiles. Among

TABLE 3

DISTRIBUTION OF DEATHS FROM TUBERCULOSIS OF WHITE PERSONS (BUFFALO, 1949–1951) BY ECONOMIC QUARTILE AT DEATH AND AT EARLIEST ADDRESS FOUND

Economic Quartile at Death	Economic Quartile of Earliest Address					
	I	II	III	IV	N.A.*	Total
Males†						
I (highest)	16	10	4	3	6	39
II	13	23	12	10	2	60
III	8	12	24	17	6	67
IV (lowest)	2	13	17	134	42	208
N.A.	1	—	—	—	5	6
Total	40	58	57	164	61	380
Females‡						
I (highest)	10	3	1	1	2	17
II	6	13	1	6	2	28
III	1	6	12	8	2	29
IV (lowest)	—	3	7	24	2	36
N.A.	—	—	—	—	3	3
Total	17	25	21	39	11	113

* N.A. = not ascertained.

† Chi-square (difference between early and death quartile distribution) = 1.37, $0.80 > P > 0.70$.

‡ Chi-square = 2.38, $P = 0.50$.

TABLE 4

DISTRIBUTION OF DEATHS FROM TUBERCULOSIS OF WHITE PERSONS (BUFFALO, 1949–1951) TRACED TO 1925–1935 BY ECONOMIC QUARTILE AT DEATH AND AT EARLIEST ADDRESS FOUND

Economic Quartile at Death	Economic Quartile of Earliest Address				
	I	II	III	IV	Total
Males*					
I (highest)	14	7	4	2	27
II	12	16	9	10	47
III	6	11	20	12	49
IV (lowest)	2	12	15	92	121
Total	34	46	48	116	244
Females†					
I (highest)	7	3	—	1	11
II	3	11	1	6	21
III	1	4	8	4	17
IV (lowest)	—	3	6	22	31
Total	11	21	15	33	80

* Chi-square = 2.06, $0.70 > P > 0.50$.
† Chi-square = 0.37, $P = 0.95$.

those traced back the farthest, to 1925–1935, the findings were the same as for the entire group (table 4).

Similar analysis by different age groups gives essentially the same results (table 5).

DISCUSSION

It was not possible to find any positive evidence for the theory that the excess of deaths from tuberculosis in the low income groups is the result of downward socio-economic "drift" caused by the disease itself. The data in tables 3, 4, and 5 show no significant differences between the quartile distributions at death and at the earliest address found, even when the subjects have been traced back for periods of fifteen to twenty-five years.

There was a disproportionately large number of males in quartile IV who could not be traced however; that is, 20 per cent as compared with about 8 per cent in the other three quartiles combined (*see* table 3). The untraced males in quartile IV differed from those traced in that they included significantly more single persons and persons born in states other than New York. No significant differences were found in age distribution or occupational class.

It is possible that the males in quartile IV who were not traced were persons who drifted into quartile IV from a higher socio-economic status. If this assumption is made and the excess (24 of the 42 males) is assigned equally to quartiles I, II, and III, there would be 48 males in quartile I, 66 in quartile II, 65 in quartile III, and 164 in quartile IV. This distribution is significantly different from the distribution at time of death.

TABLE 5

DISTRIBUTION OF DEATHS FROM TUBERCULOSIS OF WHITE PERSONS (BUFFALO, 1949–1951) IN DIFFERENT AGE GROUPS, BY ECONOMIC QUARTILE AT DEATH AND AT EARLIEST ADDRESS FOUND

Economic Quartile at Death	Age at Death: 10 to 49 Years Economic Quartile of Earliest Address					
	I	II	III	IV	N.A.*	Total
Males†						
I (highest)	5	3	1	1	2	12
II	4	10	2	1	1	18
III	3	6	11	3	1	24
IV (lowest)	—	6	3	35	12	56
N.A.	—	—	—	—	1	1
Total	12	25	17	40	17	111
Females‡						
I (highest)	6	2	1	1	1	11
II	4	9	1	5	1	20
III	1	4	8	7	2	22
IV (lowest)	—	2	4	14	—	20
N.A.	—	—	—	—	1	1
Total	11	17	14	27	5	74

Economic Quartile at Death	Age at Death: 50 Years or Older Economic Quartile of Earliest Address					
	I	II	III	IV	N.A.	Total
Males§						
I (highest)	11	7	3	2	4	27
II	9	13	10	9	—	41
III	5	6	13	14	5	43
IV (lowest)	2	7	14	99	30	152
N.A.	1	—	—	—	4	5
Total	28	33	40	124	43	268
Females‖						
I (highest)	4	1	—	—	1	6
II	2	4	—	1	1	8
III	—	2	4	1	—	7
IV (lowest)	—	1	3	10	2	16
N.A.	—	—	—	—	1	1
Total	6	8	7	12	5	38

* N.A. = not ascertained.
† Chi-square = 6.08, $0.20 > P > 0.10$.
‡ Chi-square = 4.56, $0.30 > P > 0.20$.
§ Chi-square = 2.39, $P = 0.50$.
‖ Chi-square = 0.63, $0.90 > P > 0.80$.

If a less extreme assumption is made, the 24 excess males are distributed among all four quartiles and the distribution among the quartiles becomes: I, 46; II, 64; III, 63; and IV, 170. This distribution is not significantly different from that at time of death.

Although in the first case the distribution resulting from reassigning the untraced individuals does differ significantly from the distribution at time of death, there are still more than three times as many deaths in the lowest as in the highest quartile. In the second case there are nearly four times as many deaths in the lowest as in the highest quartile.

These manipulations of the data do not entirely satisfy the lack of knowledge concerning the untraced persons. It is possible, although highly improbable, that all 42 of those not traced in quartile IV were originally in the higher quartiles, and it is likewise possible that all of those not traced in this and the other quartiles were originally in quartile IV. Any method of reassigning the untraced cases is speculative. The fact remains, however, that no matter which method is used, the socio-economic differences are of such magnitude that downward "drift" cannot account for them to any significant extent.

Another difficulty in the present study relates to the possibility that the tracing did not go back far enough. It is possible that in tuberculosis the downward change in socio-economic status occurs early enough in adult life so that the change is not picked up by the method used. There were only 33 white males who were traced to the age of eighteen or younger. Their quartile distribution at time of death was I, 4; II, 6; III, 6; and IV, 17 in comparison with a distribution at the time of earliest address of I, 3; II, 8; III, 5; and IV, 17. Clearly there are no significant differences in these distributions; however, the number of persons is quite small and the possibility still exists that, if all of the deaths from tuberculosis were traced back to adolescence or earlier, a different picture would emerge.

Consideration was given to the possibility of using addresses on the birth certificates of those persons who were born in Buffalo. This was not done, however, because, as stated earlier, census tracts were first set up in Buffalo in 1930 and any assignment to economic quartiles of addresses for the period prior to 1925 would be of doubtful validity.

Summary

Deaths of resident white tuberculous persons in Buffalo in 1949–1951 were found to be inversely correlated with socio-economic status as measured by economic quartile of residence. Data on the previous residences of these 493 persons were obtained; 84 per cent were traced to 1946 or earlier, and 66 per cent were traced to 1935 or earlier. The socio-economic distribution at the earliest address found did not differ significantly from the distribution at the time of death; this held true even for those traced to 1925–1935.

There was an excess of untraceable males in the lowest economic quartile; this group differed significantly in having more single persons and persons from other states than the group of lowest quartile males who were traced. It was pointed out, however, that, even when the extreme assumption was made that the untraced males in the lowest economic quartile originated in higher economic groups and they were assigned to these groups, the resultant effect on the socio-economic differences was relatively small.

It is recognized that this study does not provide a definitive answer to the question at issue since the method used did not permit a lifetime review of socio-economic status. It can only be concluded that, within the limitations of the study method, no positive evidence was obtained that the excess of tuberculosis mortality in low socio-economic areas is due to downward socio-economic "drift" by the persons afflicted with the disease.

SUMARIO

La Validez de las Diferencias Socio-económicas en la Mortalidad Tuberculosa

Las muertes de los tuberculosos de raza blanca residentes en la ciudad de Búffalo en 1949–1951 resultaron hallarse en correlación inversa al estado socio-económico, medido este por el factor económico de la residencia. Se obtuvieron datos relativos a las residencias de estas 493 personas; en 84 por ciento se trazó el paradero hasta 1946 o antes, y en 36 por ciento hasta 1935 o antes. La distribución socio-económica en la primera residencia descubierta no discrepó mayor cosa de la observada en la fecha de la muerte; esto reza hasta con los seguidos hasta 1925–1935.

Hubo un exceso de varones perdidos de vista en el sector económico más bajo; este grupo discrepó notablemente en contar con más solteros o personas procedentes de otros estados que el grupo de

varones del sector más bajo cuya residencia anterior fué localizada. No obstante, señálase que, aun cuando se aceptó la suposición extremada de que los varones intrazables del sector económico más bajo procedían de grupos económicos más altos y se les asignaba a éstos, el efecto ejercido sobre las diferencias socio-económicas era relativamente pequeño.

Reconócese que este estudio no ofrece una respuesta definitiva al punto debatido, dado que el método usado no permitió llevar a cabo un repaso del estado socio-económico durante toda la vida. Sólo cabe, pues, deducir que, dentro de las limitaciones del método de estudio, no se obtuvieron datos positivos de que el exceso de mortalidad tuberculosa en las zonas socio-económicas bajas se deba a una tendencia socio-económica descendente de parte de las personas afectadas por la enfermedad.

Resume

La valeur des différences socio-économiques dans la mortalité par tuberculose

La mortalité des personnes de race blanche, habitants de Buffalo de 1949 à 1951 est inversement proportionnelle aux conditions socio-économiques, lesquelles ont été évaluées d'après la distribution résidentielle. On a obtenu des renseignements sur les résidences précédentes de ces 493 personnes; 84% purent être suivis jusqu'en 1946 ou avant, et 66% furent suivis jusqu'en 1935 ou avant. La distribution socio-économique de l'adresse précédente découverte ne fut pas notablement différente de la distribution à l'époque de la mort; ceci s'est réellement montré exact même pour ceux qui ont été suivis jusqu'en 1925–1935.

Il y eut un plus grand nombre d'hommes sans traces dans la catégorie la plus basse au point de vue économique; ce groupe est nettement différent, ayant plus de célibataires et des personnes venant d'autres états, comparé au groupe d'hommes de la plus basse classe économique qui purent être suivis. Il fut néammoins signalé que, même lorsque l'on assuma que les hommes non suivis, dans le groupe de pauvre condition économique, venaient de groupes sociaux plus élevés, et qu'ils furent mis dans ces derniers groupes, cela n'affecta pas beaucoup les différences socio-économiques.

Il est reconnu que cette étude n'apporte pas une réponse définitive à la question étudiée, vu que la méthode utilisée n'a pas permis de considérer le statut économique des sujets pendant toute leur vie. On peut seulement conclure que, dans les limites de la méthode d'étude, on n'est pas arrivé à mettre en évidence que la plus grande mortalité par tuberculose dans les milieux économiquement bas, est due à la décadence socio-économique des personnes affligées de cette maladie.

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VACCINATION AGAINST TUBERCULOSIS WITH NONLIVING VACCINES[1, 2]

II. Vaccination of Guinea Pigs with Phenol-killed Tubercle Bacilli

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INTRODUCTION

The history of vaccination against tuberculosis with nonliving vaccines, which has been reviewed in a preceding communication (1), points unmistakably to the high immunizing efficacy of tubercle bacilli killed by a variety of physical and chemical agents. Their protective efficacy has frequently been shown to be of the same order as that of living attenuated vaccines. Several fractions derived from living and killed tubercle bacilli have also been shown to have similar protective ability. The safety of nonliving vaccines and the absence of undesirable local manifestations following their intradermal introduction in experimental animals and man have been repeatedly demonstrated, and a number of investigators have found certain immunogenically active preparations to be free of tuberculin skin-sensitizing properties.

It has nevertheless been held consistently by a large number of workers in the United States, France, Great Britain, and other countries, that acquired immunity against tuberculosis can be bestowed prophylactically only by a living vaccine. An attempt has been made in the preceding communication (1) to explain this gap between experimentally established fact and common medical opinion. It was suggested that one of the prominent reasons for the disparagement of nonliving vaccines is the fact that these are occasionally found to be ineffective in experiments in which parallel groups of animals vaccinated with living attenuated tubercle bacilli evince a definite degree of protection. It was also pointed out, however, that the validity of many comparisons of living and nonliving vaccines is often more apparent than real. By their nature, vaccines whose efficacy depends on the elaboration *in vivo* of immunogenic material by a living, and often self-reproducing, organism are likely to be effective under a greater variety of experimental circumstances than those whose activity must depend on the introduction of a limited quantity of a nonreplicable material. In the latter instance, faulty methods of preparation can irrevocably destroy biologic activity, and dose and route of administration are of major importance. In case of the former, even a small number of living bacilli, introduced into tissues by any of a variety of possible routes, can be expected eventually to produce at least a partially effective amount of immunogenically active substance. It certainly does not follow from this that living vaccines possess any inherent superiority, but it does appear that the chances of demonstrating an equal degree of protective efficacy for nonliving preparations depend to a larger degree on the availability of sufficient numbers of experimental animals to permit the establishment, empirically, of the optimal conditions of their administration.

Studies of this nature, employing the mouse as the experimental animal, were initiated some years ago in the laboratories of the Rockefeller Institute. The results of these experiments again showed the marked degree of immunogenicity and the low toxigenicity of several nonliving preparations of attenuated and avirulent tubercle bacilli (2–5). It appeared desirable to repeat and extend these studies in guinea pigs, in order to demonstrate once more the efficacy of nonliving

[1] From the laboratories of the late A. Q. Wells at the Sir William Dunn School of Pathology, University of Oxford, Oxford, England, and the Department of Bacteriology, University of California, Berkeley.

[2] This study was supported in part by the Medical Research Council of Great Britain, and in part by funds from Grant E-2309, National Institutes of Health, U.S.A. Some of the experiments were carried out during the tenure of a Merck Senior Postdoctoral Fellowship.

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[4] The greater part of these studies was initiated and carried out jointly with Dr. Wells before his untimely death on October 9, 1956. The manuscript was written subsequently, however, and any criticisms directed at the organization, presentation, or interpretation of these experiments must be borne entirely by myself.—D.W.W.

vaccines in another species. Moreover, the studies would make possible an investigation of the sensitizing qualities of nonliving vaccines in an animal in which skin hypersensitivity can be observed with greater ease and accuracy than is possible in the mouse, even by the foot-pad inoculation technique (6, 7).

The purpose of this communication is to report the results of vaccination of guinea pigs with intact, phenol-killed tubercle bacilli under a variety of conditions of vaccination and challenge infection. Phenol was used as a killing agent because a number of workers have reported the immunogenic activity of tubercle bacilli so killed (1), and because it was thought desirable to compare the reaction of guinea pigs and mice to a similarly prepared vaccine. Instead of BCG, however, virulent *human* tubercle bacilli were employed in these experiments for the preparation of killed vaccines because of several references in the literature (1) to the immunogenic superiority of killed virulent over killed attenuated bacilli. A subsequent report (8) will deal with studies in which methanolic extracts and other fractions of tubercle bacilli were employed as vaccines.

Experimental Methods

Vaccines: The human tubercle bacilli employed for the preparation of killed vaccines were cultures of the "PN," "DT," and "C" strains used for the production of tuberculin at the Veterinary Laboratories of the Ministry of Agriculture, Food and Fisheries at Weybridge.[5] The bacilli were grown in synthetic Dorset medium containing 10 per cent glycerol and 1.4 per cent asparagine, and were adjusted to a pH of 6.8. The medium was distributed in 1,000-ml. quantities in Blake bottles and inoculated after sterilization with several "flakes" of the pellicular growth of seed cultures on a similar medium. The cultures were incubated for five weeks at 37°C., at which time a heavy pellicle had formed. The medium was then removed by vacuum suction, and the remaining pellicular growth in each flask was resuspended in 200 ml. of 2 per cent phenol solution. After standing in phenol for twenty-four hours, with frequent manual agitation, equal numbers of cultures of the three strains were pooled. The bacterial mass was then removed by filtration through sterile muslin cloth, washed three times with 500-ml. quantities of distilled water and three times with similar quantities of cold acetone, air-dried, and stored over a desiccant in the cold until use. Attempts to culture the phenolized bacilli on a variety of media proved negative, and guinea pigs injected intraperitoneally and intramuscularly with quantities up to 25 mg., *dry* weight, failed to develop tuberculous disease. Microscopic examination of the phenol-killed preparation revealed it to consist largely of intact acid-fast bacilli and some bacillary fragments and debris, most of which also retained acid-fastness.

The phenolized bacilli were used for vaccinating purposes within four months of the day of preparation. Immediately before use, the appropriate quantity was added to a sterile isotonic saline solution and the mixture was ground for several minutes with a Teflon tissue-grinder (9). The resulting suspension consisted of particles of varying size, some quite large. To prevent an uneven distribution of vaccine due to the partial precipitation of the larger particles in the syringe, only one animal was injected from each loading of the syringe, and the mixture was manually agitated before loading.

In each experiment, one group of animals was vaccinated with phenol-killed bacilli of a culture of the saprophytic *Mycobacterium phlei*, obtained from the stock collection maintained by Mr. N. Smith at the Dunn School of Pathology. This organism was grown for seven to ten days on a similar medium and phenolized, washed, and dried by the same methods as were employed with human tubercle bacilli.

Several groups of animals received phenol-killed tubercle bacilli suspended in Freund's adjuvant (10). This was prepared by mixing 1.5 parts of Arlacel A with 8.5 parts of Bayol F, and then emulsifying this mixture with aqueous suspensions of vaccine in a ratio of 1 to 1. Emulsification was achieved by repeatedly drawing up and ejecting the Arlacel-Bayol mixture and the vaccine suspension through a sterile needle and syringe. Immediately before use, the emulsion was stirred for ten minutes with a high-speed motor-driven stirrer; it then had the consistency and color of "heavy" dairy cream.

The living vaccine which was employed in all experiments for purposes of comparison was a freeze-dried preparation of BCG made at the Pasteur Institute in Paris for use in man. It was stored at 4°C. until ready for use and was reconstituted in aqueous diluent immediately before it was injected. The vaccine was employed within two months of the date of manufacture, and the viability of the preparation was ascertained by plating out in dilutions on Dubos agar medium (11) and counting the number of colonies which developed.

Animals: The animals employed were young male albino guinea pigs of the Dunkin-Hartley strain, weighing between 300 and 400 gm. at the time of vaccination. They were distributed at random into the different experimental groups and housed in metal cages in groups of 5. Their diet consisted of a standard pellet preparation, hay, fresh greens (cabbage, kale, and lucerne)

[5] Obtained through the kindness of Dr. A. B. Paterson.

and water, ad libitum. The appearance of the animals was carefully observed and recorded, and one cage-group from each vaccination group was weighed at intervals throughout the experiment. There was no evidence of intercurrent disease in any animal either before or after experimental infection. The few deaths which occurred prior to challenge infection were the result of accidental trauma during handling, or appeared to be due to vaccination with toxic doses of vaccine. All of the animals that died after challenge infection were subjected to careful post-mortem examination, including histologic study of diseased organs of sample animals from each group; in every instance extensive tuberculous lesions were seen, and in no instance was there a suggestion of other infectious disease processes.

Injection of vaccines: Vaccines were injected either intraperitoneally, in the right lower quadrant, or subcutaneously, in the inguinal region. When repeated injections were given, the sites were varied so that no more than one injection was made in the same immediate area.

Challenge infection: The Vallée strain of bovine tubercle bacilli[6] was used in all experiments. Two methods of infection were employed: intramuscular injection and inhalation of a finely dispersed spray of tubercle bacilli.

Intramuscular infection was carried out by injecting into the muscles of the left thigh 0.5 ml. of a 1:100 dilution of a seven-day-old culture grown in Dubos Tween®-albumin liquid medium (12). The number of bacilli in this inoculum was ascertained by the plate-count method.

Suspensions of tubercle bacilli used for the inhalation infection were prepared as described by Albericci and Fletcher (13). A culture of the Vallée strain was started on Dorset's egg medium from a lyophilized preparation of infected mouse spleen. Subcultures were then made on Dubos liquid Tween-albumin medium. To prepare a suspension of bacilli suitable for nebulization, a seven-day-old culture was filtered through sterile filter paper; the filtrate consisted largely of single bacilli. Immediately prior to animal exposure, total cell counts were made in a Petroff-Hauser counting chamber with dark-ground illumination, and dilutions of the suspension were plated out to obtain viable count figures. A presumptive viable count for immediate reference was arrived at on the basis of numerous previous experiments which showed the viable count to be consistently of the order of 80 per cent of the total count. On the basis of this estimate, appropriate quantities of the filtered suspension were then placed in a modified Collison atomizer attached to the inhalation apparatus.

The apparatus has been described elsewhere by Henderson (14). In brief, it consists of a metal tube connected to an air pump, forming a closed circuit. An aerosol of tubercle bacilli consisting of particles of 1 μ^3 or less in size is introduced into the sterile air stream coming from the atomizing unit, which is placed at the "input" end of the tube. Two sealable ports at the distal end permit the insertion of the snouts of a pair of guinea pigs into the interior of the cloud-filled tube. The bacterial cloud is continuously recirculated through this tube by means of a pump. Air-flow control is effected by means of critical orifices at the entrance to the tube, and the pressure inside is maintained at a slightly negative level to prevent escape of the cloud from the apparatus. Silica-gel driers at the input and output connections of the pump maintain constant humidity conditions. By means of a bypass in the tube, cloud samples for *in vitro* assay can be collected via orifice impingers containing known volumes of diluent (0.1 per cent of bovine serum albumin).

Before animals were exposed, the apparatus was operated for five minutes to permit stabilization of the cloud. The animals were then permitted to inhale the cloud for a period of one minute, and impinger samples were taken at intervals of ten to twelve minutes. The final concentrations of the bacterial clouds were calculated from the plate counts made from the impinger samples. The cloud concentrations were found to be uniform throughout the exposure time of all animals during any one run and contained, per liter of air, approximately 0.1 per cent of the number of tubercle bacilli per ml. of the starting bacillary suspension. The estimation of the lung intake and pulmonary retention of the bacilli by the animals was based on the experiments of Harper and Morton (15), using radioactive spores. For an exposure time of one minute, the estimated lung intake of guinea pigs was calculated to be 15 per cent of the cloud concentration per liter, and the estimated lung retention as 50 per cent of the estimated lung intake.

Evaluation of results: With the exception of occasional animals sacrificed to ascertain whether the inhalation method of infection was successful, the animals in all experiments were permitted to live to term after the experimental infection. The employment of survival-time prolongation as a measurement of immunity was based on the considerations that it is a much more objective index than such other criteria as the extent of tuberculous involvement of organs and that it is the only one which constitutes a direct and undisputable aspect of protection. The pitfalls of judging immunity by the extent of tuberculous disease shortly after experimental infection have already been emphasized by Opie and Freund (16), who called attention to the fact that tuberculous lesions are often more extensive for a time in previously sensitized than in control animals, but that in the case of the former they may regress and heal in the later stages of the infection. Opie and Freund concluded that "survival of animals treated with a protective agent is doubtless the best measure of their resistance."

The day of death of each animal was recorded and the median and mean survival times of each

[6] Obtained through the kindness of Dr. Cynthia H. Pierce of the Rockefeller Institute.

group calculated. The relative degree of survival prolongation bestowed by the different vaccines was evaluated by comparing the mean survival time with that of the saline-vaccinated control group in each experiment. The statistical significance of these comparisons was ascertained by *t*-tests and expressed as P values.[7]

Results

Immunogenicity

Experiment 1: Groups of 15 guinea pigs each were vaccinated with the following quantities of phenol-killed human tubercle bacilli: 1.5, 3.0, 6.0, 12.0, and 24.0 mg., intraperitoneally in saline; 3.0, 6.0, and 12.0 mg., subcutaneously in saline; 0.5, 1.0, and 3.0 mg., intraperitoneally in 0.5 ml. of Freund's Bayol-Arlacel adjuvant; and 1.0 and 3.0 mg., subcutaneously in the same adjuvant. Two groups received 3.0 and 6.0 mg., intraperitoneally in saline, of a preparation of the phenolized tubercle bacilli which had been steamed in the autoclave for thirty minutes. Another group of 15 guinea pigs received 0.5 ml. of freshly reconstituted lyophilized culture of living BCG subcutaneously; this inoculum contained 3×10^6 living bacilli, as determined by plate counts of serial dilutions of the preparation made at the time of vaccination. A control group of 15 animals was given 0.5 ml. of saline intraperitoneally. In order to ascertain whether phenol-killed acid-fast bacilli of a saprophytic strain could produce any protective immunity, still another group of 15 animals was vaccinated intraperitoneally with 1.5 mg. of phenolized *M. phlei*. Six weeks after vaccination, the animals were infected by inhalation of a spray of tubercle bacilli of the virulent Vallée strain. The estimated lung retention was 6,000 bacilli.

Three other groups of 15 guinea pigs each were vaccinated simultaneously with 3.0 mg. of phenol-killed human tubercle bacilli in Freund's adjuvant, intraperitoneally; living BCG, subcutaneously; and saline, intraperitoneally. These animals were infected by the *intramuscular* route, at the same time as the other animals, with 1.0 ml. of a dilution of tubercle bacilli of the same suspension; the number of viable bacilli in this inoculum, as calculated by the plate-count method, was 2.5 to 5.0×10^5.

Forty-five days after infection, when most of the animals still appeared to be in good condition, one animal was selected at random from each group and sacrificed, to determine whether the inhalation infection was successful in initiating extensive pulmonary disease. The tracheobronchial lymph nodes of all of the sacrificed animals were found to be enlarged and caseous, and numerous macroscopic caseous lesions were seen in the lungs. There was evidence of dissemination to the spleen and liver in most of the animals. Although the animals vaccinated with living BCG and a number of those given killed bacilli appeared to have less pulmonary and generalized tuberculosis than those vaccinated with *M. phlei* or saline alone, obviously no importance can be attached to a comparison of only one animal from each group, especially in view of the considerable variation in the response to vaccination within each group as seen from the post-infection survival times. The results of the post-mortem examination of the sacrificed animals were only taken as evidence that the method of inhalation infection employed effectively introduced large numbers of tubercle bacilli into the lungs.

The days of death of the remaining animals and the median and mean survival times are shown in table 1.[8] As seen in table 1, the mean survival time of the animals vaccinated with living BCG exceeded that of the saline control group by 55 per cent. The guinea pigs vaccinated with phenolized *M. phlei* showed no survival time increase, but a number of groups of animals which had received phenol-killed human tubercle bacilli exhibited a degree of survival prolongation of the same order as, or slightly greater than, that of the BCG-vaccinated group.

Because of the complexity of the data presented in table 1, the information is rearranged and summarized in tables 1*A*, 1*B*, and 1*C*, which show, respectively, the effects of route of challenge infection, the role of dose, vehicle and route of vaccination, and the influence of heating on the immunogenic activity of phenolized tubercle bacilli.

It may be noted in table 1*A* that the mean survival time of both the vaccinated and the control groups was considerably greater in animals challenged by the intramuscular route, despite the fact that the infecting inoculum was

[7] The writer wishes to express his gratitude to Mr. S. Peto of the Microbiological Research Establishment of the Ministry of Supply at Porton for undertaking the statistical analysis of the results.

[8] Some of the results presented in this table have already been summarized in a previous Note (17). The figures there given for the per cent increases in mean survival time over that of the saline controls are seen in several instances to be 1 per cent lower than those presented here. This is due to the fact that it was neglected in the earlier report to adjust them to the nearest integer when decimals were eliminated in the final presentation.

TABLE 1

Survival Time of Guinea Pigs Vaccinated with Phenolized Human Tubercle Bacilli and Infected Six Weeks Later

Vaccine	Dose	Route of Vaccination	Vehicle	Route of Infection	Number of Infecting Bacilli	Days of Survival of Individual Animals after Challenge Infection															Median Survival Time	Mean Survival Time	Increase of Mean Survival Time over Saline Controls	Significance Level of Comparison of Mean Survival Time of Each Group with That of Saline Controls (P)
	mg.																				*days*	*days*	*per cent*	
Phenol-killed human tubercle bacilli	1.5	IP	Saline	Inhalation	6,000 (E.R.)	S	97	103	103	106	113	114	123	123	128	134	135	142	149	156	123	123	60	<0.001
	3.0	IP	Saline	Inhalation	6,000 (E.R.)	S	71	83	84	91	96	97	97	112	113	117	117	120	139	139	97	105	36	<0.001
	6.0	IP	Saline	Inhalation	6,000 (E.R.)	S	77	81	90	94	98	101	105	109	113	113	115	131	194	201	105	116	51	<0.001
	12.0	IP	Saline	Inhalation	6,000 (E.R.)	S	80	89	92	96	97	99	110	115	120	127	131	136	155	156	110	115	49	<0.001
	24.0	IP	Saline	Inhalation	6,000 (E.R.)	P	S	51	60	71	80	84	90	91	92	95	105	105	107	139	90	90	17	0.07
Phenol-killed human tubercle bacilli	3.0	SC	Saline	Inhalation	6,000 (E.R.)	S	60	70	71	76	76	77	79	84	86	89	99	105	139	149	79	90	17	0.10
	6.0	SC	Saline	Inhalation	6,000 (E.R.)	S	63	71	88	94	94	95	107	113	113	127	136	141	151	160	107	111	44	<0.001
	12.0	SC	Saline	Inhalation	6,000 (E.R.)	S	39	56	60	68	70	76	79	79	90	92	96	98	119	174	79	85	10	0.38
Phenol-killed human tubercle bacilli	0.5	IP	Freund's adjuvant	Inhalation	6,000 (E.R.)	S	86	95	96	109	110	112	116	128	137	143	145	151	179	231	116	131	70	<0.001
	1.0	IP	Freund's adjuvant	Inhalation	6,000 (E.R.)	S	55	78	79	79	108	111	113	117	117	119	119	122	129	141	113	106	38	<0.001
	3.0	IP	Freund's adjuvant	Inhalation	6,000 (E.R.)	S	67	71	85	91	92	94	97	100	103	105	113	125	141	150	97	102	32	0.001
Phenol-killed human tubercle bacilli	1.0	SC	Freund's adjuvant	Inhalation	6,000 (E.R.)	S	76	82	83	86	90	96	97	102	103	112	112	119	145	251	97	111	44	0.01
	3.0	SC	Freund's adjuvant	Inhalation	6,000 (E.R.)	S	86	89	90	91	93	94	96	97	127	133	137	140	185	186	96	117	52	<0.001
Phenol-killed and autoclaved human tubercle bacilli	3.0	IP	Saline	Inhalation	6,000 (E.R.)	S	73	82	88	95	101	101	101	102	103	106	109	112	113	122	101	101	31	<0.001
	6.0	IP	Saline	Inhalation	6,000 (E.R.)	S	29	72	80	86	86	88	94	104	113	114	122	124	146	183	94	103	34	0.01
Phenol-killed *M. phlei*	1.5	IP	Saline	Inhalation	6,000 (E.R.)	S	60	62	69	70	76	80	81	82	88	89	89	93	93	93	81	80	4	0.48
Living BCG*	0.5 ml.	SC	—	Inhalation	6,000 (E.R.)	S	83	84	85	86	92	100	102	118	122	126	146	146	152	222	102	119	55	<0.001
Saline	0.5 ml.	IP	—	Inhalation	6,000 (E.R.)	S	56	68	68	70	72	74	74	79	80	82	85	88	93	93	74	77	—	—
Phenol-killed human tubercle bacilli	3.0	IP	Freund's adjuvant	Intramuscular	2.5–5.0 × 10^5	119	120	120	124	138	154	155	162	163	164	172	173	175	184	254	162	158	14	0.12
Living BCG*	0.5 ml.	SC	—	Intramuscular	2.5–5.0 × 10^5	118	135	142	143	146	149	156	161	167	174	185	193	194	218	266	161	170	22	0.02
Saline	0.5 ml.	IP	—	Intramuscular	2.5–5.0 × 10^5	86	86	98	112	121	128	133	144	145	158	159	162	164	188	198	144	139	—	—

IP = Intraperitoneally.
ER = Estimated lung retention of the virulent bovine strain Vallée.
S = Animal sacrificed on forty-fifth postinfection day.
P = Animal died previous to infection (traumatic injury).
SC = Subcutaneously.
* — Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6 × 10^8 living bacilli per ml.

approximately one hundred times larger than in the inhalation-infected animals. However, the *increases* in mean survival time of the vaccinated animals over those of their respective controls were more than twice as great in the inhalation-infected group. The protection elicited by the killed preparation in this comparison (3.0 mg. of phenolized human tubercle bacilli given intraperitoneally in saline) was statistically highly significant in the inhalation-infection group, but was of no significance in the intramuscularly infected one. The available facilities did not permit a comparison of the means of infection in more of the vaccination groups, and far-reaching conclusions as to the superiority of infection by spray inhalation for the demonstration of the protective activity of vaccines cannot be drawn from this experiment. However, the present observations were corroborated in other trials (8) and were therefore deemed sufficient ground to employ inhalation-infection exclusively in all subsequent experiments of this study.

The effects of dose, vehicle, and route of vaccination are presented in table 1*B*. When given by the intraperitoneal route, the phenolized bacilli suspended in saline provided significant survival protection over the dose range of 1.5 to 12.0 mg.; 24.0 mg. gave no protection. It is of special interest that the lowest dose employed, 1.5 mg., appeared to be the most effective, a phenomenon seen even more clearly in the series of animals in which the vaccine was given intraperitoneally in Freund's adjuvant. It thus seems possible that the smallest amount of vaccine employed in this experiment was already in excess of the optimal dose; indeed, other unpublished experiments have indicated that the optimal immunogenic dose of phenol-killed tubercle bacilli for guinea pigs may be below 1.0 mg., dry weight, when administered intraperitoneally. The maximally effective dose of this vaccine is thus seen to be of the same order in the guinea pig as in the mouse (3, 4), and actually appears to be somewhat lower for the former. When the vaccine was injected subcutaneously in saline, the effective range was a narrower one: 3.0 and

TABLE 1*A*

COMPARISON OF INHALATION AND INTRAMUSCULAR ROUTES OF INFECTION

Vaccine	Route of Infection	Mean Survival Time	Per Cent Increase of Mean Survival Time over Respective Saline Controls	Significance Level of Comparison (P)
		days		
Phenol-killed human tubercle bacilli, 3.0 mg., IP*	Inhalation	105	36	<0.001
Living BCG, 0.5 ml., S.C.†	Inhalation	119	55	<0.001
Saline, 0.5 ml., IP	Inhalation	77	—	—
Phenol-killed human tubercle bacilli, 3.0 mg., IP	Intramuscular	158	14	0.12
Living BCG, 0.5 ml., SC	Intramuscular	170	22	0.02
Saline, 0.5 ml., IP	Intramuscular	139	—	—

* IP = intraperitoneally.
† SC = subcutaneously.

TABLE 1*B*

EFFECT OF DOSE, VEHICLE AND ROUTE OF VACCINATION ON IMMUNIZING EFFICACY OF PHENOL-KILLED HUMAN TUBERCLE BACILLI

Phenol-killed Human Tubercle Bacilli Suspended in:	Dose	Per Cent Increase of Mean Survival Time over Saline Controls	
		Intraperitoneal Vaccination	Subcutaneous Vaccination
	mg.		
Saline	1.5	60	—
Saline	3.0	36	17
Saline	6.0	51	44
Saline	12.0	49	10
Saline	24.0	17	—
Freund's adjuvant	0.5	70	—
Freund's adjuvant	1.0	38	44
Freund's adjuvant	3.0	32	52

TABLE 1*C*

EFFECT OF AUTOCLAVING ON IMMUNIZING EFFICACY OF PHENOL-KILLED HUMAN TUBERCLE BACILLI

Dose of Phenol-killed Human Tubercle Bacilli, Intraperitoneally	Per Cent Increase of Mean Survival Time over Saline Controls	
	Bacilli Autoclaved after Phenolization	Bacilli not Autoclaved after Phenolization
mg.		
3.0	31	36
6.0	34	51

12.0 mg. provided no protection, but 6.0 mg. was almost as efficacious in prolonging survival time as the similarly administered living BCG. When the subcutaneously injected killed vaccine was emulsified in adjuvant, however, even smaller doses—1.0 and 3.0 mg.—were effective.

The results presented in table 1*C* indicate that intensive heating had very little, if any, effect on reducing the immunogenic activity of the phenol-killed preparation under the prevailing experimental conditions. However, in view of the reports of some investigators that high temperatures do destroy such activity (1), the possibility must be considered that this effect might be dependent on other experimental variables.

Considerable importance is attached by some investigators to weight changes of animals after tuberculous infection as an index of resistance. This may be a misleading criterion of immunity, however, in experiments in which considerations of space and cost do not permit the *individual* accomodation and weighing of animals. Thus, in the present studies, with the animals housed and weighed in groups of 5, it was found that the death of one or more in a cage almost invariably resulted in a rapid and marked rise in the average weight of the remainder. This appeared to be due largely to the elimination of emaciated, early-dying animals whose presence had depressed the average weight of the group, but it also occasionally appeared that more food was subsequently ingested by the survivors (although every attempt was made to always keep the supply of all dietary items in excess of consumption). Average weight increases arising from such causes are obviously artifacts of calculation or environment, and may give an impression of resistance quite inconsistent with survival prolongation or other criteria of immunity. Weight fluctuations are therefore likely to be accurate reflections of immunity only as long as no deaths have occurred in the weighed group.

A comparison is made in table 2 of mean survival time increases of the *entire vaccination groups* with mean weight changes of the *weighed subgroups* during the second month following infection, when deaths had not yet taken place, but some of the animals ceased to gain weight.

The data presented in table 2 show that the animals vaccinated with living BCG continued to gain weight during the first seven weeks after infection at the same rate as a similarly vaccinated but uninfected group. In contrast, animals which had been given *M. phlei* or saline alone lost weight after the first month. The guinea pigs which had received killed vaccines that provided a better than 30 per cent increase in mean survival time continued to gain weight during this period. On the other hand, two of the three groups which received killed preparations providing no significant survival prolongation ceased to gain weight after the middle of the second post-infection month. The third of these groups, vaccinated with 24.0 mg. of phenolized bacilli intraperitoneally, gained weight for seven weeks, at the end of which one animal died. The two groups in which the survival prolongation elicited by killed vaccines was greater than that given by living BCG—1.5 mg. in saline or 0.5 mg. in Freund's adjuvant, intraperitoneally—showed weight gains of the same order as that of the BCG-vaccinated group throughout the observation period. Only one other group exhibited a similar consistent weight gain: the animals vaccinated intraperitoneally with 1.0 mg. of killed bacilli in Freund's adjuvant, whose mean survival prolongation was 38 per cent. Thus, while the data presented in table 2 do not speak for a direct relationship between degree of survival prolongation and post-infection weight change in every instance, they do indicate that a consistent weight increase through the second month after infection constitutes a good prognosis for an increased longevity.

It has been shown in the past by a number of investigators that repeated injections of some nonliving antituberculous vaccines are more efficacious than a single vaccination and, also, that the length of the vaccination-challenge interval may be an important limiting factor. Experiments were therefore designed to study the duration of the immunity bestowed by phenolized vaccines and the effects of their repeated administration.

Experiment 2: Groups of 15 guinea pigs each were vaccinated by three or six injections of 1.0 mg. each of phenol-killed human tubercle bacilli. The interval between injections was one week for animals receiving a total of six and two weeks for those receiving only three injections. A number of groups received the vaccine suspended in saline, and others in Freund's adjuvant. The subcutaneous or intraperitoneal route of injection was employed. Control groups of animals, similar to those described in the previous experiment, re-

TABLE 2

RELATIONSHIP OF SURVIVAL TIME INCREASE AND WEIGHT CHANGE AFTER INFECTION IN GUINEA PIGS VACCINATED WITH PHENOL-KILLED TUBERCLE BACILLI

Vaccine	Per Cent Increase of Mean Survival Time over Saline Controls	Mean Weight* 1 Month after Infection	Changes in Mean Weight* at Following Subsequent Intervals			
			1 week	2 weeks	3 weeks	4 weeks
		gm.				
Phenol-killed human tubercle bacilli						
1.5 mg., IP,† in saline	60	610	+15	+70	+60	+70
3.0 mg., IP, in saline	36	675	−5	+5	+25	+15
6.0 mg., IP, in saline	51	710	+10	+40	+30	+10
12.0 mg., IP, in saline	49	630	+10	+40	+20	+20
24.0 mg., IP, in saline	17	585	−5	+35	+35	1 animal dead
Phenol-killed human tubercle bacilli						
3.0 mg., SC‡ in saline	17	610	−10	+20	0	−10
6.0 mg., SC, in saline	44	625	+15	+25	+15	+15
12.0 mg., SC, in saline	10	550	+10	0	−30	2 animals dead
Phenol-killed human tubercle bacilli						
0.5 mg., IP, in Freund's adjuvant	70	630	−5	+40	+40	+40
1.0 mg., IP, in Freund's adjuvant	38	700	+15	+70	+40	+40
3.0 mg., IP, in Freund's adjuvant	32	750	−35	+30	+10	+20
Phenol-killed human tubercle bacilli						
1.0 mg., SC, in Freund's adjuvant	44	660	0	+30	+10	0
3.0 mg., SC, in Freund's adjuvant	52	700	0	+40	+40	+50
Living BCG,§ 0.5 ml., SC	55	760	−10	+40	+40	+60
Phenol-killed *M. phlei*, 1.5 mg., IP, in saline	4	610	−10	−20	−40	1 animal dead
Saline, 0.5 ml., IP	—	630	−15	0	−10	−30
	Not infected but weighed at same times					
Living BCG,§ 0.5 ml., SC	—	750	+20	+40	+60	+110

* Mean weight of subgroup of 5 animals in each vaccination group of 15.

† IP = intraperitoneally.

‡ SC = subcutaneously.

§ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

ceived single injections of living BCG, phenol-killed *M. phlei*, or saline. Twelve weeks after the first vaccine injection, all animals were infected by inhalation of a spray of the virulent Vallée strain of tubercle bacilli giving an estimated lung retention of 6,000 bacilli. The survival data are presented in table 3.

It may again be seen in table 3 that phenol-killed *M. phlei* in the quantity here employed had no protective activity, while both the living and nonliving vaccines succeeded in significantly prolonging survival time. One preparation of the latter—3 subcutaneous injections of 1.0 mg. of phenol-killed bacilli in Freund's adjuvant—provided a slightly superior degree of survival protection than living BCG. It does not appear, however, that repeated injections raised the immunogenic activity of killed vaccines to a categorically higher order, although the possibility that this would have been the case had the vaccination-challenge interval been a shorter one cannot be disregarded.

The importance of choosing the optimal route of vaccination for each vaccine preparation is clearly indicated in this instance: the increase in mean survival time was 79 per cent when the three injections of vaccine in adjuvant were by the subcutaneous route, and only 43 per cent when injected by the intraperitoneal one. This finding demonstrated that the most successful route of vaccination with killed vaccines is not necessarily the most directly systemic (and for practical purposes, the least feasible for human vaccination).

In the following experiment, the vaccination-challenge interval was extended still further, and the effect of repeated versus single injection was studied in several comparable vaccination groups in the same experiment.

Experiment 3: Groups of 15 guinea pigs each were vaccinated either by single injections of 3.0 or 6.0 mg. of phenol-killed tubercle bacilli, or by three injections, at two-week intervals, of 1.0 mg. each. The vaccines were given either by the sub-

TABLE 3

SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH PHENOL-KILLED HUMAN TUBERCLE BACILLI AND INFECTED 12 WEEKS LATER BY INHALATION OF 6,000 BACILLI (ESTIMATED LUNG RETENTION) OF THE BOVINE VALLÉE STRAIN

Vaccination				Days of Survival of Individual Animals after Challenge Infection															Median Survival Time	Mean Survival Time	Increase of Mean Survival Time over Saline Controls	Significance Level of Comparison of Mean Survival Time of Each Group with That of Saline Controls (P)
Vaccine	Dose and Schedule	Route	Vehicle																			
																			days	*days*	*per cent*	
Phenol-killed human tubercle bacilli	3 × @1.0 mg. at 2-week intervals	IP*	Saline	P‡	P	45	49	61	61	63	68	69	71	74	75	78	87	90	68	69	64	<0.001
	6 × @1.0 mg. at 1-week intervals	IP	Saline	P	P	40	45	55	55	58	67	67	68	68	70	71	74	84	67	63	50	<0.001
	6 × @1.0 mg. at 1-week intervals	SC†	Saline	36	39	41	42	44	48	49	51	52	61	69	71	82	111	112	51	61	45	0.009
	3 × @1.0 mg. at 2-week intervals	IP	Freund's adjuvant	45	48	51	52	53	56	58	58	59	60	63	66	69	79	86	58	60	43	<0.001
	3 × @1.0 mg. at 2-week intervals	SC	Freund's adjuvant	44	59	59	59	61	61	66	71	73	77	82	83	85	99	143	71	75	79	<0.001
Phenol-killed *M. phlei*	1 × @1.5 mg.	IP	Saline	25	29	29	31	34	36	38	39	40	42	42	46	63	65	66	39	42	0	—
Living BCG§	0.5 ml.	SC	—	45	52	54	57	58	59	60	66	70	74	82	83	91	102	109	66	71	69	<0.001
Saline	0.5 ml.	IP	—	31	32	36	36	37	38	40	41	41	41	45	48	50	54	60	41	42	—	—

* IP = intraperitoneally.

† SC = subcutaneously.

‡ P= Animal died previous to infection (traumatic injury).

§ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^8 living bacilli per ml.

TABLE 4

SURVIVAL TIME OF GUINEA PIGS VACCINATED WITH PHENOL-KILLED HUMAN TUBERCLE BACILLI AND INFECTED 36 WEEKS LATER BY INHALATION OF 9,000 BACILLI (ESTIMATED LUNG RETENTION) OF THE BOVINE VALLÉE STRAIN

Vaccination				Days of Survival of Individual Animals after Challenge Infection																Median Survival Time	Mean Survival Time	Increase of Mean Survival Time over Saline Controls	Significance Level of Comparison of Mean Survival Time of Each Group with That of Saline Controls (P)
Vaccine	Dose and Schedule	Route	Vehicle																				
																				days	*days*	*per cent*	
Phenol-killed human tubercle bacilli	1 × @3.0 mg.	IP*	Saline	25	26	26	30	34	35	35	35	38	38	40	42	44	61	62		35	38	41	0.003
	3 × @1.0 mg. at 2-week intervals	IP	Saline	29	31	31	34	35	36	38	39	41	41	44	46	50	58	66		39	41	52	<0.001
	1 × @3.0 mg.	SC†	Saline	20	22	23	24	25	26	28	29	29	30	30	34	40	42	46		29	30	11	0.24
	3 × @1.0 mg. at 2-week intervals	SC	Saline	18	23	24	26	27	27	27	27	29	30	37	39	40	51	90		27	34	26	0.15
	1 × @6.0 mg.	IP	Saline	P‡	27	29	29	29	30	31	31	32	35	37	39	44	50	51		31	35	30	0.004
	1 × @6.0 mg.	SC	Saline	21	22	22	22	22	23	24	24	24	25	26	27	29	41	48		24	27	0	—
	3 × @1.0 mg. at 2-week intervals	IP	Freund's adjuvant	P	22	22	23	26	26	26	30	31	36	37	39	42	43	57		30	33	22	0.07
	3 × @1.0 mg. at 2-week intervals	SC	Freund's adjuvant	P	27	29	30	34	35	36	39	40	40	42	47	48	52	61		39	40	48	<0.001
Phenol-killed *M. phlei*	1 × @1.5 mg.	IP	Saline	20	20	21	21	21	21	22	22	23	24	25	26	28	28	32		22	24	−15	—
Living BCG§	0.5 ml.	SC	—	27	29	33	34	35	38	38	40	41	42	47	56	56	59	61		40	42	56	<0.001
Saline	0.5 ml.	IP	—	P	P	20	22	22	22	24	24	24	25	27	29	34	34	40		24	27	—	—

* IP = intraperitoneally.

† SC = subcutaneously.

‡ P = Animal died previous to infection (traumatic injury).

§ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

cutaneous or intraperitoneal route, and were suspended in saline or in Freund's adjuvant. Control groups were given single injections of living BCG, phenolized *M. phlei*, or saline. Thirty-six weeks after the initial vaccine injection, all animals were infected by inhalation of a spray of the virulent Vallée strain of tubercle bacilli providing an estimated lung retention of 9,000 bacilli. The survival data are presented in table 4.

As seen in table 4, the mean survival times of all vaccination groups were considerably less in this than in the preceding experiments, probably because of the greater number of infecting tubercle bacilli. The relative *increases* in survival time of animals vaccinated with living BCG and with some of the killed preparations were of the same order, however, as those observed previously. The phenol-killed *M. phlei* not only failed to prolong, but actually somewhat shortened, the survival time of the animals.

Repeated injections of the killed vaccines appeared to be slightly more effective than single injections. Thus, three intraperitoneal injections of 1.0 mg. of phenol-killed human tubercle bacilli each prolonged the survival time of the animals 52 per cent; when a single injection of 3.0 mg. was given, the increase was only 41 per cent. When the route of vaccination was subcutaneous, a single vaccination of 3.0 mg. provided no protection, as was also the case when the vaccination-challenge interval was only six weeks (*see* table 1); multiple injections totaling the same quantity of vaccine bestowed some degree of survival prolongation. The differences in mean survival time between singly and repeatedly vaccinated animals were not statistically significant, however.

The superiority of the subcutaneous to the intraperitoneal route of vaccination when the killed vaccine was suspended in Freund's adjuvant was again indicated: three injections of 1.0 mg. each by the subcutaneous route increased the mean survival time by 48 per cent, whereas the same regimen of vaccine given intraperitoneally provided only a 22 per cent increase. When the vaccine was administered in saline, on the other hand, the intraperitoneal route was more effective, whether it was given by single or repeated injection.

The results of *Experiment 3* show that killed as well as living vaccines are, on the whole, capable of eliciting a degree of survival protection even when the vaccination-challenge interval represents a considerable portion of the normal life expectancy of the animals. At the same time, killed vaccine administered under *certain* circumstances appeared to be effective only when challenge infection followed vaccination after a relatively short space of time. Thus, 6.0 mg. of

TABLE 5

EFFECT OF VACCINATION-CHALLENGE INTERVAL ON IMMUNOGENICITY OF PHENOL-KILLED HUMAN TUBERCLE BACILLI

(Comparison of All Comparable Vaccine Groups)

Vaccine	Per Cent Increase of Mean Survival Time over Respective Saline Controls of Animals Infected at the Following Intervals after Vaccination:		
	6 weeks	12 weeks	36 weeks
Phenol-killed human tubercle bacilli			
3.0 mg., IP,* in saline	36	—	41
6.0 mg., IP, in saline	51	—	30
3.0 mg., SC,† in saline	17	—	11
6.0 mg., SC, in saline	44	—	0
Phenol-killed human tubercle bacilli			
3 × @ 1.0 mg., IP, in saline	—	64	52
3 × @ 1.0 mg., IP, in Freund's adjuvant	—	43	22
3 × @ 1.0 mg., SC, in Freund's adjuvant	—	79	48
Phenol killed *M. phlei*, 1.5 mg., IP, in saline	4	0	−15
Living BCG,‡ 0.5 ml., SC	55	69	56

* IP = intraperitoneally.

† SC = subcutaneously.

‡ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

phenol-killed tubercle bacilli injected subcutaneously in saline prolonged the mean survival time 44 per cent when the vaccination-challenge interval was six weeks (table 3), but gave no protection when it was thirty-six weeks.

The effects of the vaccination-challenge interval on the duration of the immunogenic activity of living BCG and phenol-killed vaccines are summarized in table 5, which shows the increases in survival time of comparably vaccinated groups of *Experiments 1*, *2*, and *3*. Whereas the phenol-killed preparation was generally less effective as the time permitted to elapse between vaccination and infection was increased, such loss of efficacy with time was not always the case. This is illustrated by the group of animals vaccinated intraperitoneally with a single injection of 3.0 mg. of phenolized tubercle bacilli in saline: the increase in mean survival prolongation was 36 per cent when the vaccination-challenge interval was six weeks and 41 per cent when it was thirty-six weeks. Because of this divergence and the small number of comparable vaccination groups infected at different intervals, conclusions concerning the duration of the immunity elicited by killed vaccines cannot be made. It is apparent even here, however, that the duration of a significant degree of immunity provided by phenol-killed vaccines under at least some circumstances of administration is not appreciably shorter than that elicited by living BCG. This is shown by the fact that the relative increase in mean survival time of some groups of animals vaccinated with the killed preparations was still of BCG-order when challenge took place almost nine months after vaccination.

Toxigenicity

Experiments conducted in mice have already provided some evidence for the separateness of the immunogenic and toxigenic qualities of phenol-killed tubercle bacilli (2, 3, 18, 19). Thus, variations in the methods of preparation and subsequent treatment of killed vaccines were found to affect individually their ability to immunize and to cause toxic reactions; the toxigenic and immunogenic properties of phenolized preparations of substrains of the same organism were shown to vary independently; and fractions of tubercle bacilli which possess only one or the other of these properties to any extent have been isolated. Other investigators have reported essentially similar findings (1), and it is quite clear, at least from the recent literature, that the elicitation of heightened resistance against tuberculosis by killed vaccines is not necessarily accompanied by post-vaccinal intoxication.

Nevertheless, here again, popular medical opinion seems to hold otherwise, possibly because of reports in the early literature that intact killed tubercle bacilli are not well tolerated by tissues. It also appears that the fervent insistence by the proponents of living BCG on the absolute safety of their vaccine has given rise in the minds of many to the corollary, but erroneous, supposition that *only* living BCG is fully safe. The first of these considerations has been discussed in the previous communication (1): In summary, although intact tubercle bacilli, living or dead, do indeed evoke local and even systemic symptoms of irritation and toxicity, the amounts of many killed bacillary preparations necessary to bestow a high degree of immunity are much too small to cause any readily discernible evidence of toxicity. This is true especially of those killed preparations from which at least some of the toxic, nonimmunogenic lipids are removed. The second consideration does not constitute a bona fide argument, and can only be met by the repeated demonstration that living BCG is *not* the only safe vaccine. This is again shown by the data summarized in tables 6 and 7.

A record of the post-vaccination appearance, incidence of death, and weight changes of the animals vaccinated with the various preparations described in *Experiments 1 and 2* is presented in table 6. As has been mentioned above, only one subgroup of 5 animals from each vaccination group of 15 was weighed throughout the experiment, but the appearance of the animals and the incidence of deaths in the entire vaccination groups were recorded. It was found consistently that, when weight loss did occur after vaccination, it was greatest at the end of the first post-vaccination week. The mean weight changes on the seventh post-vaccination day are therefore recorded in table 6; the weight changes on the thirty-seventh day are also presented to indicate the later weight characteristics of the animals.

It is apparent from table 6 that very few animals became fatally ill as a result of the vaccination, even when as much as 24.0 mg., *dry*

weight, were given intraperitoneally. The few deaths which occurred were all among intraperitoneally injected animals and, with one exception, among those which received repeated administrations of vaccine. Although visceral adhesion formation was usually seen at postmortem examination of these animals, this did not appear to be sufficiently extensive to account for death. Instead, death appeared to be the outcome of a generalized peritonitis, which may

TABLE 6

WEIGHT CHANGE AND APPEARANCE OF GUINEA PIGS FOLLOWING VACCINATION WITH PHENOL-KILLED TUBERCLE BACILLI

Vaccine	Mean Weight* One Hour before Vaccination	Change in Mean Weight* at:		Deaths† during Vaccination-Infection Interval	Physical Appearance‡ during Vaccination-Infection Interval
		7 Days after Vaccination	37 Days after Vaccination		
	gm.	*gm.*	*gm.*		
Phenol-killed human tubercle bacilli 1.5 mg., IP,§ in saline	330	+65	+230	0	Normal
3.0 mg., IP, in saline	320	+40	+250	0	Normal
6.0 mg., IP, in saline	325	+35	+185	0	Normal
12.0 mg., IP, in saline	320	+5	+230	0	Slight lethargy during first 7 days
24.0 mg., IP, in saline	320	−20	+70	1‖	Lethargic, emaciated, dull for first 4 weeks, then recovered
Phenol-killed human tubercle bacilli 3.0 mg., SC,¶ in saline	320	+50	+230	0	Normal
6.0 mg., SC, in saline	310	+50	+220	0	Normal
12.0 mg., SC, in saline	310	+50	+240	0	Normal
Phenol-killed human tubercle bacilli 0.5 mg., IP, in Freund's adjuvant	370	+20	+170	0	Normal
1.0 mg., IP, in Freund's adjuvant	385	+20	+175	0	Normal
3.0 mg., IP, in Freund's adjuvant	375	+5	+185	0	Normal
1.0 mg., SC, in Freund's adjuvant	380	+55	+220	0	Normal
3.0 mg., SC, in Freund's adjuvant	400	+30	+200	0	Normal
3 × @ 1.0 mg., phenol-killed human tubercle bacilli, IP, in saline	320	+50	+120	2**	Lethargic, emaciated, dull for first 6 weeks, then recovered
6 × @ 1.0 mg., phenol-killed human tubercle bacilli, IP, in saline	320	+20	—††	2‡‡	Same general condition as above for 6 weeks, then recovered
3 × @ 1.0 mg., phenol-killed human tubercle bacilli, SC, in saline	330	+50	+220	0	Normal
6 × @ 1.0 mg., phenol-killed human tubercle bacilli, SC, in saline	390	+60	+170	0	Normal
3 × @ 1.0 mg., phenol-killed human tubercle bacilli, IP, in Freund's adjuvant	310	+10	+40	0	Lethargic, emaciated, dull for first 6 weeks, then recovered
3 × @ 1.0 mg., phenol-killed human tubercle bacilli, SC, in Freund's adjuvant	305	+55	+235	0	Normal
Phenol-killed *M. phlei*, 1.5 mg., IP, in saline	400	+20	+200	0	Normal
Living BCG,§§ 0.5 ml. SC	340	+60	+250	0	Normal
Saline, 0.5 ml., IP	380	+60	+230	0	Normal

* Mean weight of subgroup of 5 animals in each vaccination group of 15.

† Deaths out of entire vaccination group of 15.

‡ Based on physical appearance of all animals in each vaccination group.

§ IP = intraperitoneally.

‖ Died 13 days after vaccination: generalized peritonitis.

¶ SC = subcutaneously.

** Died 26 and 27 days after vaccination, respectively: generalized peritonitis.

†† Weight accidentally not taken.

‡‡ Died 20 and 48 days after vaccination, respectively: generalized peritonitis in first animal; second died after traumatic injury in handling.

§§ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

TABLE 7

COMPARISON OF IMMUNOGENICITY AND TOXICOGENICITY OF VACCINE PREPARATIONS

Vaccine	Per Cent Increase of Mean Survival Time over Respective Saline Controls (Arranged in Decreasing Order)	Mean Weight Change* 7 Days after Vaccination	Deaths† during Vaccination-Infection Interval	Appearance‡ during Vaccination-Infection Interval
Vaccination-Challenge Interval 6 weeks				
		gm.		
Phenol-killed human tubercle bacilli 0.5 mg., IP,§ in Freund's adjuvant	70	+20	0	Normal
1.5 mg., IP, in saline	60	+65	0	Normal
Living BCG,‖ 0.5 ml., SC¶	55	+60	0	Normal
Phenol-killed human tubercle bacilli 3.0 mg., SC, in Freund's adjuvant	52	+30	0	Normal
6.0 mg., IP, in saline	51	+35	0	Normal
12.0 mg., IP, in saline	49	+5	0	Slightly ill for 7 days
6.0 mg., SC, in saline	44	+50	0	Normal
1.0 mg., SC, in Freund's adjuvant	44	+55	0	Normal
1.0 mg., IP, in Freund's adjuvant	38	+20	0	Normal
3.0 mg., IP, in saline	36	+40	0	Normal
3.0 mg., IP, in Freund's adjuvant	32	+5	0	Normal
24.0 mg., IP, in saline	17	−20	1**	Ill for 4 weeks
3.0 mg., SC, in saline	17	+50	0	Normal
12.0 mg., SC, in saline	10	+50	0	Normal
Phenol-killed *M. phlei* 1.5 mg., IP, in saline	4	+20	0	Normal
Vaccination-Challenge Interval 12 weeks				
3 × @ 1.0 mg. phenol-killed human tubercle bacilli, SC, in Freund's adjuvant	79	+55	0	Normal
Living BCG,‖ 0.5 ml., SC	69	+60	0	Normal
3 × @ 1.0 mg. phenol-killed human tubercle bacilli, IP, in saline	64	+50	2††	Ill for 6 weeks
6 × @ 1.0 mg. phenol-killed human tubercle bacilli, IP, in saline	50	+20	2‡‡	Ill for 6 weeks
6 × @ 1.0 mg. phenol-killed human tubercle bacilli, SC, in saline	45	+60	0	Normal
3 × @ 1.0 mg. phenol-killed human tubercle bacilli, IP, in Freund's adjuvant	43	+10	0	Ill for 6 weeks
Phenol-killed *M. phlei*, 1.5 mg., IP, in saline	0	+20	0	Normal
Weight Control				
Saline, 0.5 ml., IP	—	+60	0	Normal

* Mean weight of subgroup of 5 animals in each vaccination group of 15.

† Deaths out of entire vaccination group of 15.

‡ Based on physical appearance of all animals in each vaccination group.

§ IP = intraperitoneally.

‖ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

¶ SC = subcutaneously.

** Died 13 days after vaccination: generalized peritonitis.

†† Died 26 and 27 days after vaccination, respectively: generalized peritonitis.

‡‡ Died 20 and 48 days after vaccination, respectively: generalized peritonitis in first animal; second died after traumatic injury in handling.

have been the manifestation of a local hypersensitivity reaction or could have developed as a result of the introduction of contaminating organisms with the vaccines (which were not sterilized after preparation). In most of the vaccination groups, however, the weight gain and appearance of the animals after vaccination remained normal. When signs of illness or retardation of weight gain were seen, they were almost invariably transient.

In table 7, the protective efficacy of the several vaccines described in *Experiments 1* and *2* is compared with their effects on the weight and appearance of the animals after vaccination. It is clear at a glance that survival prolongation after infection and weight-gain retardation after vaccination did not go hand in hand. Weight gain and appearance after vaccination with some of the most effectively administered preparations were of the same order as in the saline-injected controls, and a marked retardation of weight gain was seen more often in groups whose vaccination provided only a low degree of survival protection.

In evaluating "toxicity" in these experiments, no recourse was made to detailed histologic examination of the tissues of vaccinated animals. Reliance was placed entirely on weight changes and general appearance and behavior to indicate any grossly inimical properties of the vaccines. This is admittedly a very crude evaluation of toxicity. However, the appearance of the organs of the few animals that died accidentally during handling after vaccination with the killed vaccine revealed no abnormalities, and this has been corroborated in other, unpublished experiments in which sample animals of effectively vaccinated groups were sacrificed at intervals after vaccination and the gross appearance of their organs examined. In contrast, it was found that living BCG of the same origin and in quantities similar to those employed here frequently induced the formation of fairly extensive visceral lesions which persisted for some months after vaccination (20). Preliminary as these observations are, when taken together with the fact that nonliving vaccines cannot induce progressive tuberculous disease, whereas living BCG may do so under certain physiologic or pathologic conditions (21, 22), they do point to nonliving ones as being more desirable from the point of view of safety.

Allergenicity

The animals in the survival experiments reported here were not tuberculin tested after vaccination, to avoid any possible effects which the introduction of tuberculin, the evocation of a hypersensitivity reaction, or even the mere process of further injection might have on their resistance to tuberculosis. A separate experiment was therefore undertaken to determine the tuberculin-sensitizing capacity of small quantities of the same preparation of phenol-killed tubercle bacilli as was employed for vaccination purposes in *Experiments 1, 2,* and *3.*

Four groups of 8 guinea pigs each were vaccinated with one of the following: 0.2 or 0.8 mg. of phenol-killed human tubercle bacilli intraperi-

TABLE 8

SKIN REACTIVITY OF GUINEA PIGS TO PPD (0.2 ML. OF A 1:10 DILUTION) 5 WEEKS AFTER VACCINATION WITH PHENOL-KILLED HUMAN TUBERCLE BACILLI AND LIVING BCG

Vaccine	Maximal Average Diameter Induration* at Site of Tuberculin Injection of Each Animal							
	mm.							
Phenol-killed human tubercle bacilli, 0.8 mg., IP,† in saline	10	11	13	14	14	18	19	20
Phenol-killed human tubercle bacilli, 0.2 mg., IP, in saline	5	11	11	11	12	14	15	16
Living BCG,‡ 0.5 ml. SC§	14	16	18	19	20	21	24	28
Saline, 0.5 ml. IP	0	0	0	0	1	1	1	2

* During observation period of 24 to 48 hours after tuberculin injection.

† IP = intraperitoneally.

‡ Reconstituted suspension of a freshly obtained lyophilized preparation, made at the Pasteur Institute, Paris, found to contain 6×10^6 living bacilli per ml.

§ SC = subcutaneously.

toneally in 0.5 ml. of saline; 0.5 ml. of a reconstituted suspension of the lyophilized Pasteur BCG, containing 6×10^6 living bacilli per ml. subcutaneously; or 0.5 ml. of saline, intraperitoneally. Five weeks after vaccination, all animals were injected intradermally in a shaved area of the abdomen with 0.2 ml. of a 1:10 dilution of concentrated Weybridge mammalian PPD. The reactions at the site of injection were observed for forty-eight hours.

The maximal average diameter of induration which developed at the site of the PPD injection during the forty-eight hours after testing is presented in table 8. It may be seen in this table that 0.8 mg. of the phenolized preparation caused the development of a degree of tuberculin skin hypersensitivity not much lower than that elicited by living BCG (although the reactions of fewer animals vaccinated with the killed preparation in this and other unpublished experiments progressed to necrosis). The smaller quantity of killed vaccine, 0.2 mg., was still sufficient to produce a degree of hypersensitivity in all animals and, as will be indicated in a subsequent publication, even smaller amounts frequently evoked a mild degree of tuberculin skin hypersensitivity. The animals in *Experiments 1*, *2*, and *3*, which received quantities of phenol-killed tubercle bacilli 1.5 mg. or greater, must therefore be considered to have been sensitized by the vaccination.

Discussion

The results obtained from these experiments show, once more, that nonliving vaccines can be at least as effective against tuberculosis as living ones, but that their efficacy varies with the conditions under which they are administered and tested. The reasons why some investigators have in the past failed to demonstrate a significant degree of immunizing activity by killed bacillary preparations would appear to be quite clear in the light of these findings. Dosage, route and vehicle of administration, the length of the vaccination-challenge interval, and the method of challenge infection were all found to affect the demonstration of immunogenic behavior by phenol-killed tubercle bacilli, and experiments in which the optimal experimental conditions are not ascertained empirically are not unlikely to lead to a negative evaluation.

Neither the killed nor the living vaccines provided a very high degree of protection in the present study, which in this respect corroborates the observations of most other investigators. Thus, Irvine wrote in 1934 that "all investigators are agreed that it is impossible completely to immunize the guinea-pig against tuberculosis; the most that can be done is to delay the progress of the disease so that the inoculated animal lives longer than the control" (23). In a later review of the efficacy of living BCG in the guinea pig, the same writer again concluded that the effects of vaccination are only manifested in a delay of death after infection, and that the degree of this survival prolongation varied in the hands of different workers (24).

The protection elicited by living BCG in the present experiments was lower than that reported by some, but of the same order as that reported by many other workers. For example, the extent of survival prolongation of guinea pigs reported in 1938 by Schwabacher and Wilson (25) is almost identical with the values obtained here, and Schwabacher and Wilson cite a large number of other studies showing essentially similar results. Quantitative differences reported for the immunizing capacity of BCG can be accounted for not only on the basis of subtle differences in vaccine preparations, but undoubtedly also arise from variations in the methods of vaccination and experimental infection, the nature of the test-animal population, its diet, care, and accommodation, and other environmental variables. In the present case, the living BCG preparation appeared to be satisfactory: it has been tested widely in animals and employed extensively in man, and viability analyses immediately before use confirmed a large number of viable bacilli to be present in the inocula. It is likely, therefore, that the extent of its activity here observed is in large part a reflection of the particular conditions of the test situation. Had these circumstances been favorable for the demonstration of a somewhat greater immunizing efficacy by living BCG, there is no reason to assume, therefore, that the nonliving vaccines would not also have shown correspondingly greater activity. Support for this contention comes from the many previously reported studies (1) in which killed vaccines have been found to be as effective as living ones even when the degree of resistance enhanced by the latter was more dramatic than that observed here.

In choosing the methods of vaccination and

infection, consideration was given to the avoidance of experimental artifacts. The vaccines were given by intraperitoneal or subcutaneous injection, although neither of these routes would be practical for human vaccination, and the latter usually resulted after some weeks in the formation of suppurating vaccinal lesions when living BCG or phenol-killed tubercle bacilli in excess of 3.0 mg. were given. Intradermal inoculation, which is much less likely to produce persistent local reactions, was not employed here because primary importance was placed on exactness of dosage, and this route is likely to give rise to inaccuracies: a part of the vaccine inoculum is lost not infrequently through immediate leakage, or through early expulsion with sloughed-off tissue should even a superficial lesion develop. While further experiments to test the efficacy of intradermally injected phenol-killed vaccines in the guinea pig, in comparison with living BCG, are desirable, it should be recalled that killed vaccines have already been shown by a number of investigators to bestow significant protection on experimental animals and man when administered by the intradermal route (1).

The manner of challenge infection, direct inhalation of a cloud of tubercle bacilli, was dictated by the desire to approach as nearly as possible the events characteristic of the most common pathway of human infection with tubercle bacilli, at least in countries where pasteurization of milk or tuberculin testing of dairy herds has been widely accepted. Moreover, challenge by intramuscular injection into the thigh or inguinal region, the route of infection commonly employed in tuberculosis studies with the guinea pig, frequently results in the development of massively destructive, crippling lesions *in situ*, and it was believed that this artifact, relative to naturally occurring tuberculosis, is likely to obscure the true picture of systemic host resistance. The superiority of inhalation over injection as a means of infection for the assay of acquired immunity was here indicated experimentally.

Using the mouse as the experimental animal, it was noted (4) that the immunogenic activity of certain fractions of tubercle bacilli could be demonstrated most clearly when challenge infection was small and the course of the disease consequently chronic, i.e., conditions resembling those of natural infection of man and other animals. It would be desirable, therefore, to repeat the present experiments, employing those conditions of vaccination found to be most effective, but infecting the animals much more lightly. Albericci has shown that an estimated lung retention of 30 bacilli (as based on Poisson distribution probability) suffices to initiate an eventually fatal tuberculous disease in every exposed normal guinea pig (26). Even smaller numbers of virulent bacilli have been found by other workers to be uniformly fatal to guinea pigs. The difficulty that has so far stood in the way of testing comparatively the activity of killed vaccines and living BCG in such minimally infected animals is the problem of housing large numbers of guinea pigs for long periods of time.

There has been considerable interest during recent years in the "nonspecific" immunologic properties of bacteria and some of their components. In a given host-parasite relationship this interest divides itself into two areas of investigation: The question posed by the one is whether the resistance-enhancing or resistance-reducing reactions elicited in the host by a component of the parasite are manifested only vis-a-vis that particular parasite, or whether they have implications that extend to other parasitic relationships. The question suggested by the other is whether the known immunologic role played by a particular bacterial moiety in a disease initiated by that organism can be duplicated by substances derived from another organism (or even by nonmicrobial substances).[9] In tuberculosis there appear to be elements of "nonspecificity" in both areas. Thus, on the one hand, certain extracts of tubercle bacilli have been shown to affect the resistance of experimental animals to other infections (e.g., 27, 28), and vaccination with intact killed tubercle bacilli has, recently, even been claimed to reduce the incidence of poliomyelitis

[9] It may be stated in passing that the fact that components of certain microorganisms may evoke host responses whose effects go beyond immediate and specific attempts at defense is not surprising, in view of the profound influence which one infectious relationship is often known to have on another, concomitant one. The ability to make such "nonspecific" immunologic effects is by no means limited to materials of microbial origin and, indeed, a consideration of the wide range of substances and conditions which can affect the interplay of host and parasite robs the concept of "nonspecific" immunity produced by a bacterial fraction of some of its connotations of novelty and uniqueness.

in children (29). The evocation of "nonspecific" immunologic activity by tubercle bacilli is clear, moreover, from their well-established property of modifying and enhancing immunologic responses to unrelated antigens. It has also long been recognized that the tuberculous subject differs in his susceptibility to other infectious agents (30). On the other hand, experiments in mice have shown that substances of nonmycobacterial origin—for example, the endotoxins of gram-negative bacteria, pertussis vaccine, and even mineral oil—can under certain experimental circumstances affect to some degree the survival time of animals after challenge infection (5, 31, 32).

To clarify further the degree of specificity (both of origin and of scope) of the immunity elicited by killed preparations of tubercle bacilli against tuberculosis, experiments were designed along two lines: an inquiry into the effects of such vaccines on resistance to other pathogens and an examination of the possibility of producing a similar degree of resistance to tuberculosis by material not derived from tubercle bacilli. The results of the former approach will be communicated at a later time. The results of preliminary experiments toward the latter have already been indicated above. On the assumption that a vaccine prepared from a taxonomically closely related but immunologically distinct organism would be most likely to have some protective activity, animals in each experiment were vaccinated intraperitoneally with 1.5 mg. of the saprophytic *M. phlei*, grown, killed, and prepared in a way similar to the preparation of the human tubercle bacilli. While the latter, given in the same quantity and by the same route, provided protection of the same degree as elicited by living BCG, the *M. phlei* preparation was consistently and entirely devoid of immunogenic activity. It is not impossible that a vaccine made from this or similar organisms would show some activity at a different dose level, but it would appear from the present study that killed *tubercle bacilli* (and phenol- or heat-killed preparations of such other strains of tubercle bacilli as H37Ra, BCG-Phipps, and BCG-Tice have also been found to have immunogenic activity [3, 4, 31]) possess immunogenic powers against tuberculosis in the guinea pig which distinguish them at least quantitatively from phylogenetically very close microorganisms. It would be of interest to determine whether the taxonomically very distantly removed substances reported by Dubos and Schaedler (32) to have immunogenic activity against tuberculosis in mice can elicit similar effects in the guinea pig.

Safety of vaccination, in the sense that a vaccine will not initiate the progressive disease which it is intended to prevent, is inherently absolute in any killed vaccine. Safety as it refers to the absence of any pathologic sequelae of vaccination, however, is not necessarily always assured. The toxigenic properties of phenol-killed tubercle bacilli were therefore investigated, and the results speak for a wide margin of safety between the maximally effective and the minimally toxic (grossly) doses.[10] If animal experiments here serve as a guide, the large practical problem of possible future human vaccination with phenol-killed tubercle bacilli appears to be one of establishing the maximally effective dose, and not one of concern over injurious effects likely to be produced by such, or even considerably larger, quantities.

The amount of killed bacilli which elicited the highest degree of immunity observed in these experiments was surprisingly small and is of the same order as the optimal dose for the mouse. The difficulty of predicting the activity of the vaccine in a new species on a body weight-dose

[10] It is possible that the decrease in efficacy of the vaccine with increases in dosage even to levels still below that at which physical symptoms of illness appeared indicates a threshold area of toxicogenicity, at which the toxic effects of a preparation suffice to prevent or nullify its immunogenic ones, but do not cause gross pathologic symptoms. This may, in fact, explain why the intraperitoneal route of administration, superior to the subcutaneous one when the vaccine was suspended in saline, appeared less satisfactory when it was emulsified in oil adjuvant: the adjuvant enhances toxicogenicity as well as immunogenicity (3) and intraperitoneal introduction of a mildly toxic material is more likely to be inimical. Another explanation for the lack of immunogenic activity of quantities of the preparation below the threshold of gross toxicity is suggested by the observations of Schaedler and Dubos (33) and others, that cellular constituents of some microorganisms can bring about, in certain circumstances, an exacerbation of chronic or latent heterologous infections. Such an event is likely to place a considerable extra burden on the defense mechanisms of an individual, and may consequently obscure the potentially immunogenic effects of a vaccine.

relationship thus becomes obvious, and the optimal quantity of killed antituberculous vaccines for man must probably also be ascertained empirically in each case. The initial difficulties posed by this may be considerable, but they appear small in comparison with the permanent problems inherent in vaccination with an unstable, not entirely innocuous, living organism such as BCG.

The arguments in favor of a killed vaccine have been discussed at length previously (1), and it is sufficient to say here that the experimental results obtained again offer every indication that killed vaccines should be given serious attention for employment in man. Although the phenol-killed preparation here tested proved to be at least as effective as living BCG, there is no reason to assume that it is the *most* effective, or otherwise the most satisfactory, vaccine. The advantages inherent in its nonviability may provide enough cause to warrant its consideration for human trial even now, but it would be very much more desirable if a preparation possessing at least the same degree of efficacy and safety, but devoid of tuberculin-sensitizing properties, could be made available. Work toward this end will be described in a forthcoming communication (8).

Summary

A vaccine prepared from phenol-killed human tubercle bacilli was found to be at least as effective, under certain experimental conditions, as living BCG in prolonging the survival time of guinea pigs infected by inhalation of a cloud of virulent tubercle bacilli.

The degree of survival protection bestowed by the killed vaccine varied with the dosage, the route and vehicle of administration, and the length of the vaccination-challenge interval. The preparation was most effective when very small quantities were used: a single intraperitoneal injection of 1.5 mg. suspended in saline, or of 0.5 mg. suspended in Freund's adjuvant, provided a somewhat better protection than living BCG. When the killed bacilli were suspended in adjuvant, the subcutaneous route of vaccination appeared to be more effective than the intraperitoneal one.

Under certain conditions of administration, the duration of the heightened resistance elicited by the killed vaccine was of the same order as that evoked by living BCG even when a period of almost nine months elapsed between vaccination and challenge.

The killed vaccine caused no gross manifestations of toxicity in quantities at which protection was elicited. Even in considerably larger amounts, its toxic action was almost invariably mild and transient.

A similarly prepared and killed vaccine made from the saprophytic *Mycobacterium phlei* was consistently devoid of any protective activity.

Sumario

La Vacunación contra la Tuberculosis con Vacunas no Vivas: II. La Vacunación de los Cobayos con Bacilos Tuberculosos Matados con Fenol

Una vacuna preparada de bacilos tuberculosos humanos matados con fenol resultó ser a lo menos tan eficaz, en ciertas condiciones de experimentación, como el BCG vivo, en lo tocante a prolongar el tiempo de sobrevivencia de los cobayos infectados por inhalación de una nube de bacilos tuberculosos virulentos.

La intensidad de la protección de la sobrevivencia otorgada por la vacuna matada varió de acuerdo con la dosis, la vía y el vehículo de administración y la duración del intervalo transcurrido entre la vacunación y la provocación. La preparación alcanzó su mayor eficacia cuando se usaron cantidades muy pequeñas: una sola inyección intraperitoneal de 1.5 mg. suspendida en solución salina o de 0.5 mg. suspendida en el coadyuvante de Freund otorgaron una protección algo mejor que la brindada por el BCG vivo. Cuando se suspendieron los bacilos muertos en el coadyuvante, la vía subcutánea de vacunación resultó más eficaz que la intraperitoneal.

En ciertas condiciones de administración, la duración de la resistencia realzada que provocara la vacuna muerta fué del mismo orden que la provocada por BCG vivos, aun cuando transcurrió un período de casi nueve meses entre la vacunación y la provocación.

La vacuna muerta no ocasionó mayores manifestaciones de toxicidad en cantidades que facilitaban protección. Aun en cantidades mucho mayores, la acción tóxica fué casi invariablemente leve y pasajera.

Una vacuna preparada en forma semejante y matada, elaborada del *Mycobacterium phlei*, se mostró constantemente desprovista de toda facultad protectora.

Resume

Vaccination contre la tuberculose avec des germes tués. II. Vaccination de cobayes avec des bacilles tuberculeux tués par le phénol

L'auteur a trouvé qu'un vaccin préparé à partir de bacilles tuberculeux humains tués par du phénol était aussi efficace, dans certaines conditions expérimentales, que le BCG vivant pour prolonger la vie de cobayes infectés par inhalation d'un nuage de bacilles tuberculeux virulents.

Le degré de protection leur permettant de survivre à l'infection, grace au vaccin de germes tués, varie avec la dose, la voie et l'excipient administrés et la longueur de l'intervalle entre la vaccination et l'attaque par les bacilles. La préparation fut très efficace quand on utilisa des très petites quantités: une seule injection intrapéritonéale de 1,5 mg en suspension saline de 0,5 mg en suspension dans l'excipient de Freund, procura une protection quelque meilleure que le BCG vivant. Quand les bacilles tués furent mis dans cette dernière suspension la voie sous-cutanée de vaccination apparut plus efficace que la voie intrapéritonéale.

Dans certaines conditions d'administration, la durée de résistance accrue, apportée par le vaccin de germes tués, fut du même ordre que celle donnée par le BCG vivant, même lorsqu'il y eut une période de près de neuf mois entre la vaccination et l'infection expérimentale.

Le vaccin fait avec des germes tués ne cause aucune majeure manifestation de toxicité aux quantités nécessaires pour apporter une protection. Même avec des quantités considérablement plus importantes, son effet toxique fut presqu' invariablement léger et transitoire.

Un vaccin préparé de la même façon avec des germes tués de *Mycobacterium phlei* fut constamment dépourvu d'activité protectrice.

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THE INFLUENCE OF CORTICOSTEROID HORMONES IN THE TREATMENT OF TUBERCULOUS MENINGITIS IN NEGROES[1]

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INTRODUCTION

Isoniazid, streptomycin, and PAS are well established as the main antimicrobial agents in the treatment of tuberculous meningitis. While mild cases of tuberculous meningitis respond favorably to antimicrobial treatment alone, in advanced cases the mortality rate is still very high. Therefore, the search for new treatment methods and for auxiliary drugs has continued.

Smith and Vollum (1) have shown that intrathecal administration of PPD may promote the resolution of gelatinous exudate that causes cerebrospinal block. Such treatment, however, is very laborious, time-consuming, requires extensive laboratory and some neurosurgical facilities, is distressing to the patient, is potentially dangerous (2, 3), and is by no means uniformly successful.

In 1951, L. W. Kinsell (4) first suggested the use of corticosteroid hormones in the treatment of tuberculous meningitis. Since then numerous favorable reports have appeared in the literature (5–9). The hormones have been given systemically and intrathecally. In this country, the majority of physicians treating tuberculous meningitis agree that the corticosteroid hormones are a valuable adjunct to the antimicrobial agents, particularly when cerebrospinal block complicates the picture (10).

Because of the relative infrequency of tuberculous meningitis and its grave prognosis, randomized studies which could prove the usefulness of these hormones are rare. To the knowledge of the present writers, only a few such studies have been reported (11, 12), and in these only a few cases were compared.

The purpose of this report is to compare the results in treatment of tuberculous meningitis obtained in two groups of patients not selected at random. Nevertheless, both groups had the same basic antimicrobial regimen, and one group received steroid hormones while the other did not. These two groups have been formed as the frequency of the use of corticosteroids in tuberculous meningitis has gradually increased through the last six years.

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METHODS AND MATERIALS

Each patient, to be admitted to this study, had to fulfill the following criteria: They must have been Negroes with active meningitis who had had isoniazid and streptomycin early in the course of therapy. If streptomycin intrathecally had been used, not more than ten instillations may have been given. Early deaths unrelated to tuberculosis or its treatment were excluded. The meningitis must have been at the moderate or severe stage according to the classification by the Medical Research Council of Great Britain (1948). According to this classification there are three stages of tuberculous meningitis:

The mild stage includes patients with undisturbed consciousness and no, or only mild, focal neurologic signs.

The moderate stage includes patients with disturbed consciousness who are not comatose or delirious and those with moderate focal neurologic signs such as paraparesis, hemiparesis, and single cranial nerve involvement.

The severe stage includes comatose or delirious patients and those with severe focal neurologic signs such as paraplegia, hemiplegia, and multiple cranial nerve involvement. Only those patients have been described as comatose who did not respond to any stimuli, including pain.

By setting up these strict criteria the number of cases compared was reduced, but the homogenicity of the groups compared was enhanced. Every patient who satisfied the above requirements was included in the analysis. From June, 1952, to August, 1958, 33 such patients were admitted to Battey State Hospital and were analyzed. All received the same basic antimicrobial treatment. Sixteen of these also received corticosteroids (referred to as "steroid regimen") and 17 had only antimicrobial agents (referred to as "standard regimen").

Eleven other patients had only mild meningitis and were not included in the study. Eight received antimicrobial agents alone and the other 3 had added corticosteroid hormones. All 11 patients survived without residual damage. It is believed that the corticosteroid hormones hastened the recovery of the 3 patients in whom they were used,

TABLE 1

AVERAGE DOSE OF ANTIMICROBIAL AGENTS IN MILLIGRAMS PER KILOGRAM

Regimen	Number of Patients	Isoniazid	Streptomycin	PAS
1) Children				
Standard	7	12.7	55	545
Steroid	8	13.5	50	355*
2) Adults				
Standard	10	7.9	25	226
Steroid	8	8.7	21	208

* Average for 7 patients. One did not receive PAS.

but no objective criteria to support these observations are available.

Treatment

The dose of antimicrobial agents varied from case to case according to the stage of the disease and the weight and the age of the patient. Parenteral streptomycin was given on a daily schedule from thirty to one hundred and twenty days, usually sixty days, and was then reduced to a twice-weekly schedule for a total of six to twelve months. Four patients received intrathecal streptomycin with a maximum of eight instillations. Para-aminosalicylic acid was given orally.

The average dose of each drug in milligrams per kilogram of body weight at the time of the patients' admission to the series is given in table 1. The patients were divided into two groups according to the treatment regimen. It is evident that the dosage of each antimicrobial agent was higher in the children than in the adults. The duration of chemotherapy in cases in which it was completed varied between twenty and thirty months.

In May, 1955, cortisone was used in the treatment of tuberculous meningitis for the first time. Up to the termination point of the study (August, 1958) 16 patients who met the above requirements received corticosteroid hormones. Fifteen received cortisone by the intramuscular route at the onset of therapy. During the last eighteen months most adults received prednisone as soon as oral administration appeared feasible. One patient was started on oral prednisone. In adults the average initial dose of cortisone was 2.3 mg. per kg. of body weight ranging from 1.5 to 4.2 mg. In children the average dose was 3.2 mg. per kg. of body weight ranging from 1.25 to 5.5 mg. This dose was maintained for three to seven days, depending on the response, and was then gradually tapered off. The hormones were given for a period ranging from seven to eighty-seven days with an average duration of thirty-four days. Increased intracranial pressure, disturbed consciousness, and severe focal signs were considered main indications for continuance of hormone therapy. Relatively little attention was paid to the abnormalities of cerebrospinal fluid when duration of treatment with hormones was under consideration providing that, in cases of cerebrospinal block, the improvement was steady and the intracranial pressure was not markedly elevated.

The changes in cerebrospinal fluid were typical for tuberculous meningitis in all 33 cases included in this series. In 20 patients the tuberculous etiology was proved by culture of tubercle bacilli from the cerebrospinal fluid and in one case by autopsy. In 6 cases the supportive evidence was obtained by bacteriologically proved tuberculosis elsewhere in the body. In the other 6 patients no bacteriologic proof was obtained. One patient was treated elsewhere for miliary tuberculosis with pleural effusion, receiving 2 gm. of streptomycin daily for sixty days. The remaining 5 patients were all small children with skin reactions to tuberculin and roentgenographic evidence of primary tuberculosis.

RESULTS

The therapeutic results obtained in the two treatment regimens are given in table 3. The mortality rate among the 17 patients in the "standard regimen" was 52.9 per cent and among the 16 patients in the "steroid regimen" was 18.8 per cent.

Two relatively small groups were compared. The statistical significance of the results obtained can be ascribed to difference in treatment only if both groups are very similar in every other respect. Independent of this study, 56 cases of patients with tuberculous meningitis treated on this hospital service were carefully analyzed (13). The following factors appeared to have an ad-

TABLE 2

CORRELATION BETWEEN THERAPEUTIC REGIMENS AND PROOF OF TUBERCULOUS ETIOLOGY OF MENINGITIS

Regimen	Direct		Circumstantial	
	Tubercle Bacilli in Cerebrospinal Fluid	Tuberculous Meningitis at Autopsy	Tuberculous Disease of Other Systems	Proof of Tuberculous Infection, i.e., Tuberculin Reactors
Steroid	9 (1)	1 (1)	3	3* (1)
Standard	11 (7)	—	3	3 (2)
Total	20 (8)	1 (1)	6	6* (3)

( ) Number of patients who died.

* One patient was treated for miliary tuberculosis prior to admission; she received 2 gm. of streptomycin daily for 60 days.

verse influence on the course of the disease and on the final outcome:

1. Patients of marginal ages (up to two years and more than forty years of age) did not improve as much as the intermediate age group (two to forty years).
2. Patients with disturbances of consciousness or with focal neurologic signs (Stage 2) did not improve as much as those without either (Stage 1).
3. The most ominous single finding was coma.
4. Severe focal signs, while important, did not herald a fatal outcome as frequently as severe disturbances of consciousness.
5. Duration of symptomatic disease prior to onset of treatment appeared significant.
6. Cerebrospinal block was a serious complication and fatal in all patients who did not receive corticosteroid therapy.
7. A low cerebrospinal fluid sugar (less than 20 mg. per 100 ml.) appeared to be a sign of poor prognosis.
8. A negative cutaneous reaction to tuberculin in the face of advanced disease was found chiefly in patients with fatal outcome.
9. Severe tuberculous complications, such as miliary tuberculosis and far advanced pulmonary tuberculosis, did not appear to be decisive in the group as a whole, but were considered the primary cause of death in 2 patients and contributory in 4 of the total 12 fatalities.

These factors have been taken into consideration to determine the comparability of the two groups in the present study, and are listed according to the treatment regimen in table 4. It is apparent that both groups are comparable. However, more cases of block were found in the "steroid regimen" and more with severe pulmonary tuberculosis and negative reaction to tuberculin in the "standard regimen."

The fact that both groups as a whole compare well still does not necessarily mean that the cases in both regimens were comparable. To facilitate the comparison of individual cases, a numerical index was assigned to each significant prognostic factor. With this index, as much weight was attributed to a factor as it appeared that this particular factor had contributed to the fatal outcome of the patients who died.

After careful evaluation of the importance of each factor and after review of the available literature on the subject, the index listed in table 5 was adopted.

When the factor indices found in one patient were numerically computed, the prognostic index of this patient was determined. The maximal index for a case was 12 and the minimal 1.

In table 6 the number of cases and the fatalities in each prognostic index and in each regimen are

TABLE 3

MORTALITY: TWO REGIMENS COMPARED

Regimen	Number of Patients	Patients Who Died	Per Cent Mortality
Standard	17	9	52.9
Steroid	16	3	18.8
Total	33	12	36.4

TABLE 4

INCIDENCE OF IMPORTANT PROGNOSTIC FACTORS IN THE TWO THERAPEUTIC REGIMENS

Factors	Standard Regimen		Steroid Regimen	
	Total Number of Patients	Patients Who Died	Total Number of Patients	Patients Who Died
1. Age				
Intermediate (2–40 years)	7	3	8	1
Marginal (0–2 and 40 years)	10	6	8	2
2. Duration of symptoms prior to treatment:				
0–7 days	7	2	5	0
7–14 days	5	3	6	1
14 plus days	5	4	5	2
3. Stage of disease:				
Moderate stage	11	4	11	1
Severe stage				
Severe focal signs	3	2	2	0
Coma	2	2	0	0
Coma and severe focal signs	1	1	3	2
4. Cerebrospinal fluid:				
Sugar below 20 mg./100 ml.	3	2	4	1
Partial block	2	2	3	0
Complete block	2	2	6	1
5. Negative tuberculin reaction	3	3	1	0
6. Miliary or far advanced disease	11	6	7	1

TABLE 5

WEIGHT ASSIGNED TO FACTORS THAT INFLUENCE PROGNOSIS IN TUBERCULOUS MENINGITIS

Factor	
1. Intermediate age	0
Marginal age	1
2. Duration 0–7 days	0
8–14 days	1
14 plus days	2
3. Moderate stage	1
Severe stage	
Severe focal signs	2
Coma	3
Coma and severe focal signs	4
4. Cerebrospinal block, partial	1
Cerebrospinal block, complete	2
5. Cerebrospinal fluid sugar below 20 mg. per 100 ml	1
Negative reaction to tuberculin	1
Miliary or far advanced pulmonary tuberculosis	1

shown. The distribution of the cases by index was similar. When fatalities are compared, it may be seen that in the "steroid regimen" no patient with an index less than 6 and only one of 4 with this index died. Among the patients in the "standard regimen," 2 patients with indices as low as 3 and 4 died, and no patient with an index of 5 or more survived. In both regimens all patients with indices higher than 6 died.

It is believed that from these figures it has been shown clearly that the corticosteroid hormones were definitely beneficial in the treatment of the patients with tuberculous meningitis. From table 4 it is evident that the corticosteroids were most effective in patients with cerebrospinal block. None of 4 patients in the "standard regimen" with cerebrospinal block survived; and of 9 patients with block in the "steroid regimen," 8 survived. It may be postulated that advantage in the use of corticosteroids is confined to these cases alone.

In further analysis, the patients who had cerebrospinal block and died were excluded. Still, almost three times as many patients died on antimicrobial agents alone as when corticosteroids were used. This suggests the use of these hormones in every case of advanced tuberculous meningitis whether the cerebrospinal block is present or not.

The complications due to corticosteroids were rare. In one case, a mild Cushing syndrome developed and cleared gradually after discontinuance of hormones. In another case, complications attributed to steroids were multiple and consisted of psychosis, urinary infection, mixed peritonitis, and paralytic ileus. When cortisone was rapidly tapered off, episodes of adrenal cortical insufficiency with hypotension and shock forced the resumption of the hormone treatment in spite of existing contraindications. After a stormy clinical course, the patient recovered eventually. This case demonstrates the complications that may be encountered during treatment with cortisone. On the other hand, it is unlikely that this patient would have survived the meningitis if treated with antimicrobial agents alone.

It is beyond the scope of this paper to report in detail on the follow-up observations and quality of recovery of the patients. It should be stated, however, that there was no recurrence of meningitis when the recommended course of isoniazid was taken. The quality of recovery was excellent or at least satisfactory in all but 3 cases. One patient treated with steroids is mentally retarded. Severe sequelae have been observed in 2 cases in the "standard regimen." One is mentally deficient, a behavior problem, and has severely impaired speech. Another has paraparesis, in-

TABLE 6

CORRELATION BETWEEN TREATMENT, PROGNOSTIC INDEX, AND MORTALITY

Number of Patients

Prognostic Index	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Standard therapy	1	2	3 (1)	4 (1)	1 (1)	3 (3)	2 (2)	1 (1)
Steroid therapy		1	2	3	4	4 (1)	2 (2)	
Total	1	3	5 (1)	7 (1)	5 (1)	7 (4)	4 (4)	1 (1)

( ) Number of patients who died.

continence of the bladder, and blindness of the right eye. Thus, 2 of 8 survivors in the group treated with antimicrobials alone have severe sequelae, while of 13 survivors treated with antimicrobials and steroids only one has moderate sequelae.

Discussion

The results of treatment obtained in 33 Negro patients with moderate or severe tuberculous meningitis were analyzed.

The distribution of significant prognostic factors, such as severity and duration of the disease, age of the patient, presence of cerebrospinal block, very low cerebrospinal sugar, anergic stage, and severe pulmonary tuberculosis (miliary and far advanced), was found to be similar in both groups. However, there were more than twice as many cases of cerebrospinal block in the group treated with steroid hormones. To determine whether the distribution of prognostic factors among the patients was comparable, a numerical index was ascribed to each factor. The writers believe that, if there was any significant prognostic difference between the groups, it was to the disadvantage of the group treated with corticosteroid hormones. The mortality rate in the "steroid regimen," however, was only 18.8 per cent as compared with 52.9 per cent in the "standard regimen."

The effect of corticosteroids was most dramatic in cases of cerebrospinal block. There were 13 patients with this complication. All 4 who were treated with the "standard regimen" died, while eight of 9 receiving corticosteroids survived.

The theoretical background explaining the usefulness of corticosteroid hormones in the treatment of tuberculous meningitis should be discussed briefly.

It has been shown in many experiments and was observed repeatedly in humans that corticosteroid hormones can reduce the hypersensitivity to tuberculoproteins. This hypersensitivity is blamed for toxicity in tuberculosis, for the inflammatory response of meninges, the formation of gelatinous exudate, and for vasculitis in tuberculous meningitis. Leivac (14) has shown that endarteritis with consequent encephalomalacia is found very frequently at autopsy in children who die of tuberculous meningitis. Ebert (15) has demonstrated in his experiments on the rabbit ear chamber that cortisone reduces exudation and vasculitis in tuberculous foci. S. Feldman and collaborators (16) found that cortisone reduces or prevents the vascular changes in experimental meningitis in rabbits. Thus, it can be understood how the corticosteroid hormones may favorably influence the course of tuberculous meningitis.

According to the anatomic position of the central nervous system block, several types can be differentiated: The *arachnoid block* is characterized by marked increase of intracranial pressure due to impairment in diffusion of the cerebrospinal fluid back to the blood stream. This causes communicating (external hydrocephalus). The barrier lies in the arachnoid villi which are inflamed and practically functionless. No gelatinous exudate is necessarily involved. In this type of block, the writers believe that cortisone, in effect, is specific therapy. It is postulated that the inflammatory reaction is reduced and the normal function of the villi restored. This would increase absorption of the fluid, and also reduce exudation from the inflamed choroidal plexus and the membranes. A rapid reduction in intracranial pressure would be the usual response to treatment.

In *intracerebral block* the gelatinous exudate obstructs one of the narrow passages between the ventricles causing internal hydrocephalus. The release of the increased pressure is urgently needed. Cortisone may reduce the production of fluid but it cannot stop it; and, in the opinion of the writers, cannot resolve or aid the resolution of the exudate rapidly enough to prevent complications or even death. Surgical drainage is probably the only hopeful treatment.

Cerebrospinal or *spinal arachnoid block* develops when the exudate obstructs the foramen magnum or the spinal canal. The fluid circulates freely with the ventricles and the subarachnoid space of the cranium and can be returned to the blood stream. The intracranial pressure is not necessarily increased. On lumbar tap, the low pressure drops rapidly after the release of only a small amount of fluid. Usually, there is no response to jugular compression. Proteino-cellular dissociation is frequent. This is probably the most frequent type of cerebrospinal block and was present in all of the cases described with this complication. It is believed that in this type of block the corticosteroid hormones may be of great help.

The usual fate of the gelatinous exudate is fibrinous organization with compression of the underlying nerve tissue and centers. Depending

upon the level of the scarring, cranial nerve (particularly optic) damage, quadraplegia, paraplegia, cauda equina syndrome, or death may result. In certain experimental models, the corticosteroid hormones reduce and prevent fibroblastic proliferation (17, 18) from inflamed surfaces, and are thought to prevent the organization of the exudate. Whether this could occur in the meningeal exudate of tuberculosis is not known. In any case, this type of action would not explain the resolution of an existing exudate.

Some writers (5, 16) maintain that cortisone resolves—or at least promotes resolution of—the gelatinous exudate. Because of failure to submit a satisfactory theoretic explanation and of the difficulties in obtaining unequivocal experimental proof of such an effect, others are reluctant to recognize the usefulness of these hormones in the treatment of already present cerebrospinal block.

The present writers have an hypothesis, unaccompanied by experimental proof, that may explain the part corticosteroids play in the resolution of the exudate. Corticosteroid hormones have an inhibitory effect on fibroblasts, thereby preventing the fibrous organization of the fibrin-containing exudate. If the fibrous organization is inhibited, the body will mobilize its own repairing forces, namely fibrinolytic enzymes, which in turn will gradually resolve the exudate, restoring the normal dynamics.

It is the conclusion of the writers that there is a well-founded theoretic basis for the use of corticosteroid hormones in the treatment of tuberculous meningitis. Even more important, the experience has shown that these hormones are of great help in most clinical situations arising during treatment of this entity. Their early use is necessary in all but mild cases, if complete recovery is to be expected. Once encephalomalacia or scarring has been established, no treatment can regenerate the destroyed nerve centers.

Summary

The therapeutic results obtained in 33 Negroes with moderate and severe tuberculous meningitis were analyzed. All patients received isoniazid and parenteral streptomycin. Thirty-two received PAS and 4 had intrathecal streptomycin with a maximum of eight installations. Sixteen patients had, in addition, corticosteroid hormones.

The mortality rate among the 17 patients treated with antituberculous drugs alone was 52.9 per cent, and the rate was 18.8 per cent among the 16 who received corticosteroid hormones. In the former group, 4 patients had cerebrospinal block and all 4 died. Only one of 9 patients with cerebrospinal block who were in the "steroid regimen" died.

In order to determine whether the compared cases were prognostically similar, a prognostic index was designed. According to this index, comparable cases were present in both regimens.

An hypothesis to explain the action by which the corticosteroid hormones influenced the resolution of tuberculous exudate and helped the restitution of normal cerebrospinal fluid dynamics was offered. It was concluded that corticosteroid hormones used with isoniazid, streptomycin, and PAS are extremely valuable in the treatment of advanced cases of tuberculous meningitis, particularly when used early and in the presence of cerebrospinal block.

Sumario

El Impulso de las Hormonas Corticosteroideas en el Tratamiento de la Meningitis Tuberculosa en Sujetos de Raza Negra

Este análisis comprende los resultados terapéuticos obtenidos en 33 sujetos de raza negra que tenían meningitis tuberculosa moderada o grave. Todos los enfermos recibieron isoniacida y parentéricamente estreptomicina. Treinta y dos recibieron PAS y 4 estreptomicina por vía intratecal con un máximo de ocho instilaciones. Dieciséis enfermos recibieron además hormonas corticosteroideas.

La mortalidad entre los 17 enfermos tratados exclusivamente con drogas antituberculosas fué de 52.9 por ciento y de 18.8 por ciento entre los 16 que recibieron hormonas. En el primer grupo, 4 enfermos tenían bloqueo céfalorraquídeo y los 4 murieron. No falleció más que uno de los 9 que tenían bloqueo céfalorraquídeo en el grupo del "régimen esteroideo."

A fin de determinar si los casos comparados eran pronósticamente semejantes, se elaboró un índice pronóstico. Conforme a este índice, había casos comparables en ambos regímenes.

Se ofrece una hipótesis que explicaría la acción con la cual las hormonas corticosteroideas afectaron la resolución del exudado tuberculoso y fomentaron el restablecimiento de la dinámica normal del líquido céfalorraquídeo. Se deduce que las hormonas corticosteroideas usadas con la isoniacida, la estreptomicina y el PAS son sumamente valiosas en el tratamiento de los casos avanzados de meningitis tuberculosa, en par-

ticular cuando se usan tempranamente y si existe bloqueo céfalorraquídeo.

Resume

Importance du traitement par les hormones corticostéroïdes dans la méningite tuberculeuse chez les Noirs

Les auteurs analysent les résultats thérapeutiques obtenus chez 33 Noirs présentant des formes moyennes et graves de méningite tuberculeuse. Tous ces malades reçurent de l'isoniazide et de la streptomycine par voie parentérale. Trente deux reçurent du PAS et quatre eurent de la streptomycine intrarachidienne avec un maximum de huit injections. Seize malades eurent, en plus, des hormones corticostéroïdes.

Le taux de mortalité parmi les 17 malades traités seulement par les médicaments antituberculeux fut de 52,9% et il fut de 18,8% parmi les 16 malades qui reçurent des hormones corticostéroïdes. Dans le groupe précédent, quatre malades eurent un blocage cérébrospinal et quatre moururent. Seulement un des 9 malades avec blocage cérébrospinal et traités par les hormones corticostéroïdes mourut.

On établit un indice de pronostic pour établir si les cas que l'on comparait étaient semblable au point de vue pronostique. Selon cet indice, des cas comparables furent trouvés dans les deux catégories.

Ces auteurs avançèrent une hypothèse expliquant l'action par laquelle les hormones corticostéroïdes influençent la résorbtion de l'exsudat tuberculeux et aide la restauration d'une hydrodynamique normale du liquide céphalorachidien. Ils conclurent que les hormones corticostéroïdes, utilisées en même temps que l'isoniazide, la streptomycine et le PAS ont une grande valeur dans le traitement de cas avancés de méningite tuberculeuse surtout si on les utilise de bonne heure et quand il existe un blocage cérébrospinal.

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SICKLE CELL TRAIT AND TUBERCULOSIS[1]

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INTRODUCTION

A dispute exists in the literature over the incidence of sickle cell trait among Negro tuberculous patients (1–3). Weiss and Stecher (1) found that this particular phenomenon occurred twice as frequently among a group of patients with tuberculosis as it did in the general population. However, Rosenblum and his co-workers (2) found no such increase in the incidence of sickle cell trait among their group of tuberculous patients.

In the pathogenesis of all infectious diseases, and especially tuberculosis, individual host factors play an important role. Environmental factors, especially socio-economic factors, likewise may play a role. The difficulty in distinguishing between the respective roles of heredity and environment is well illustrated by the greater prevalence of tuberculous infection among U. S. Negroes. While this racial higher prevalence is generally explained on the basis of socio-economic and related factors, much remains unknown about the question of racial susceptibility itself. Since the influence of a hemoglobin defect upon this question remains in dispute and with the recent emphasis of the pathologic potential of sickle cell trait (4), it was deemed worth while to investigate the matter. Accordingly, it is the purpose of this paper to report the findings on the incidence of sickle cell trait among 310 Negroes who were tuberculous patients.

MATERIALS AND METHODS

Three hundred and ten Negro patients with tuberculosis had hemoglobin paper electrophoretic studies performed on oxalated venous blood samples, employing sodium barbital bath solution at a pH of 8.6 by a method modified from Singer (5). A sickle cell preparation was also done on a sample of each patient's blood by mixing it with a 2 per cent aqueous solution of sodium metabisulfite and examining the preparation for sickling phenomenon after it had stood for fifteen minutes under a cover slip, according to the method described by Ham (6). All patients were admitted to the hospital for the treatment of active tuberculosis. In 92 per cent of the cases, there was either bacteriologic or histologic proof of the diagnosis of tuberculosis. In the remaining 8 per cent, the diagnosis was based on the clinical picture, roentgenographic findings, skin tests, and response to therapy.

RESULTS

Of the 310 tuberculous patients, 28 had positive sickle cell preparations together with the characteristic AS[3] hemoglobin pattern on paper electrophoresis, an incidence of 9.0 per cent. The incidence of sickle cell trait among Negroes in the Washington, D. C. area has been shown to be 6.7 per cent based on a study of 3,000 pregnant Negro females (7). This compares favorably with the incidence of the sickling trait found in other large series concerned with investigating the Negro population at large (table 1). An analysis of the sickling trait in all reported series and in the present study in patients with active tuberculosis is reported in table 2. Also, an analysis of the 28 patients with the AS hemoglobin pattern is presented in table 3. Patients with the normal AA[4] hemoglobin pattern are shown in table 4. When examined for age at onset of disease and extent and duration of illness, there was no consistent difference between the two groups.

DISCUSSION

In a series of patients approximately twice the size of the series of Weiss and Stecher (1), it has been found that the incidence of the sickle cell trait is approximately the same in the tuberculous as in the nontuberculous Negro population. This is in contradistinction to the Weiss and Stecher

[1] From the Pulmonary Disease Division, District of Columbia General Hospital; and the Department of Medicine, Georgetown University School of Medicine, Washington, D. C.

[2] Resident Fellow, American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Hemoglobin AS represents sickle cell trait, the majority of the hemoglobin being of the normal type (A).

[4] Hemoglobin AA is normal human hemoglobin, all of the hemoglobin being of the normal type.

TABLE 1
INCIDENCE OF AS* HEMOGLOBIN IN SEVERAL LARGE GROUPS OF NEGROES

Series	Number of Patients	AS Sickle Trait
Washington, D. C. (7)	3,000	210 (6.7%)
Baltimore, Maryland (8)	500	36 (7.2%)
Galveston, Texas (9)	1,550	139 (9.0%)
Houston, Texas (10)	400	36 (9.0%)
Philadelphia, Pennsylvania (11)	1,000	74 (7.4%)
Total	6,450	495 (7.9%)

* Sickle cell trait with most of the hemoglobin of the normal type.

observation that the sicklying trait occurred in 12.6 per cent of 150 tuberculous Negroes, whereas it occurred in 5.3 per cent of 150 nontuberculous Negroes. The unusually low figure for the incidence of the sickle cell trait in their "control group" is evidence that their conclusions were derived from the study of too small a sample. The Negro population of both Philadelphia and Washington, D. C. stems from the same general area of the United States. The sickle cell trait incidence, therefore, would not be expected to vary significantly between the two groups.

Commenting on the normal incidence of sickling trait found among tuberculous Negroes in an earlier study at Sea View Hospital, Staten Island, New York (3), Weiss and Stecher suggested that there was a difference in the type of disease in patients admitted to a tuberculosis hospital as compared with disease in patients admitted to a general hospital. This had significance to them because they found that 74 per cent of those with the sickling trait had exudative tuberculosis, whereas only 38 per cent without the trait had exudative tuberculosis. One-third of the patients in the present study were in a general hospital. The incidence of sicklemia among these patients was 9.4 per cent compared with an incidence of 8.6 per cent among the remaining two-thirds of the patients in the series who were in a nearby tuberculosis hospital. These figures are certainly not significantly different.

In the only other study on the subject, Rosenblum and associates (2) found an incidence of 7.5 per cent sickle cell trait among Negroes with tuberculosis in the New York area.

When examined as to age, extent, and type of disease, there was no significant difference between the two groups in the present study. Twenty of the 28 patients with sickle cell trait were questioned closely for: (*1*) history of repeated pulmonary infections, (*2*) hemoptysis, (*3*) hematuria, and (*4*) family incidence of anemia. Three patients had two or more episodes of pneumonia in their past histories and one had had an episode of painless hematuria. Only 2 of

TABLE 2
INCIDENCE OF AS* HEMOGLOBIN IN SEVERAL GROUPS OF NEGROES WITH ACTIVE TUBERCULOSIS

Series	Number of Patients	AS Sickle Trait
Dolgopol. V., *et al.* (3)	77	3 (4.0%)
Weiss, W., *et al.* (1)	150	19 (12.6%)
Rosenblum, R., *et al.* (2)	200	15 (7.5%)
Ryan, T. J., *et al.*	310	28 (9.0%)

* Sickle cell trait with most of the hemoglobin of the normal type.

TABLE 3
AGE, EXTENT, AND TYPE OF DISEASE AMONG 28 TUBERCULOUS PATIENTS WITH AS* HEMOGLOBIN

Age Group	10–20	20–30	30–40	40–50	50–60	60–70	70+	Total
Pulmonary tuberculosis								
Minimal	0	2		2				4
Moderately advanced	0		1	4	1	2	1	9
Far advanced	0	1	2	4	4	1		12
Nonpulmonary disease								
Localized	0	0	0	3	0	0	0	3
Miliary	0	0	0	0	0	0	0	0
Total AS Patients	0	3	3	13	5	3	1	28

[5] Sickle cell trait

TABLE 4

AGE, EXTENT, AND TYPE OF DISEASE AMONG 278 TUBERCULOUS PATIENTS WITH AA* HEMOGLOBIN
(Four patients with AC† hemoglobin were excluded)

Age Group	10–20	20–30	30–40	40–50	50–60	60–70	70+	Total
Pulmonary tuberculosis								
Minimal	1	2	7	4	2	4	0	20
Moderately advanced	—	19	20	23	16	9	5	92
Far advanced	7	12	35	37	30	16	8	145
Nonpulmonary disease								
Localized	2	3	1	2	1	1	—	10
Miliary	—	3	4	3	1	—	—	11
Total AA Patients	10	39	67	69	50	30	13	278

* Normal human hemoglobin
† Hemoglobin C trait

these 20 patients had had hemoptysis. These same questions were asked of 20 control patients, matched for age, sex, and extent of disease, selected from the nonsickle cell trait group of patients. There was no significant difference between the two groups. Although these numbers are admittedly too small to be statistically significant, no trend was noted among the disease pattern of patients with sickle cell trait to suggest that the abnormality of the erythrocyte led to a greater incidence of hemoptysis, more severe infection, or a specific type of tuberculosis.

SUMMARY

Based on the study of 310 Negroes with active tuberculosis, the incidence of the AS[3] hemoglobin defect, sickle cell trait, is the same among tuberculous Negroes as it is among the general Negro population.

There is nothing noted in the disease pattern of tuberculosis as it occurs in patients with sickle cell trait to distinguish it from the disease in patients without the trait.

SUMARIO

La Característica Drepanocítica y la Tuberculosis

A base del estudio de 310 sujetos de raza negra que tenían tuberculosis activa, la incidencia de la hemoglobinopatía AS, la característica drepanocítica, es idéntica en los tuberculosos y entre la población general de raza negra.

No se notó nada en el espectro patológico de la tuberculosis tal como se observa en sujetos portadores de meniscocitos que la diferencie de la enfermedad según se observa en los sujetos sin tal característica.

RESUME

Tendance héréditaire à cellules falciformes et Tuberculose

D'après une étude portant sur 310 Noirs ayant une forme active de tuberculose, la fréquence de l'hémoglobinopathie de type AS—à cellules falciformes—est la même parmi les Noirs tuberculeux que parmi la population Noire en général.

Dans une étude détaillée de la tuberculose telle qu'elle se présente chez les malades ayant ce caractère génétique, rien ne put être retenu qui puisse la distinguer de la forme présentée par des malades n'ayant pas de cellules falciformes.

Acknowledgments

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DIFFERENTIATION OF BOVINE TUBERCLE BACILLI FROM OTHER MYCOBACTERIA BY THE DETERMINATION OF NICOTINAMIDASE ACTIVITY[1]

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INTRODUCTION

Human infection with *M. tuberculosis* var. *bovis* in the United States is no longer an important problem. In other countries, however, this is not so. Urech (1) in 1957 reported 4.5 per cent bovine infections among 441 tuberculous patients in Germany.

The major differences between the human and bovine strains are based on cultural characteristics and animal pathogenicity studies. Konno and associates (2–4) reported a biochemical method which would differentiate human tubercle bacilli from all other types of mycobacteria. This method is based on the fact that the human strains have a significantly greater niacin content than all of the other mycobacteria tested.

Halpern and Grossowicz (5) studied the amidase activities of cell-free extracts from *M. phlei* and BCG, using various substrates, and reported a higher nicotinamidase activity in *M. phlei* than in BCG. Bönicke and Lisboa (6) compared the nicotinamidase activity of bacterial emulsions of human and bovine tubercle bacilli and reported no nicotinamidase activity in the bovine variety.

The purpose of this report is to distinguish bovine tubercle bacilli from other mycobacteria (human and avian tubercle bacilli, unclassified mycobacteria, and nonpathogenic acid-fast bacilli) by determining the nicotinamidase activity of the cell-free extracts.

MATERIALS AND METHODS

Strains and culture media: Twenty-seven strains of various types of mycobacteria were used. There were 7 strains of human tubercle bacilli: H37Rv, H37Rv isoniazid-R, R1Rv, H37Ra, Aoyama B,[2] H2, and JH16Ra; 8 strains of bovine tubercle bacilli: Vallée, Ravenel, Ravenel isoniazid-R, B-4228-4, BCG-Japan, BCG-Copenhagen, and BCG-Paris; 2 strains of avian tubercle bacilli: 4110 and 4121; 4 strains of unclassified mycobacteria: P-1, P-8, P-16, and P-22;[3] 6 strains of nonpathogenic acid-fast bacilli: *M. phlei*, *M. 607*, *M. smegmatis*, *M. ranae*, guinea pig I, and guinea pig II.[4] All bacteria tested were grown on the surface of Sauton synthetic culture medium for three weeks except in the case of nonpathogenic acid-fast bacilli, which were cultured for one week only.

Preparation of cell-free extracts from mycobacteria: Bacterial pellicles (about 10 gm. wet weight) were filtered and washed with water four or five times. Washed bacteria were ground in a cold mechanical mortar with about half the amount of sea sand for thirty minutes. Then, 30 ml. of water were added and cell debris was removed by centrifuging in the Hitachi preparative ultra-centrifuge in the cold at 10,000 g. for forty minutes. Cell-free extracts were dialyzed overnight against distilled water at 6°C. The protein content of the extracts was determined by Mehl's biuret method (7).

Determination of Nicotinamidase

Quantitative method: The bacterial extracts, which contained 2 mg. of protein, served for the determination of nicotinamidase. Ten micromoles of nicotinamide in 0.05 M of pH 7.0 phosphate buffer were added to each bacterial extract to make the total volume of 2 ml. As a control, phosphate buffer and bacterial extracts without nicotinamide were used. Two tubes were placed in an incubator at 37°C. for three hours. Ammonia formed in the system was distilled by Folin's method at 50°C. into 10 ml. of N/50 H_2SO_4. Ammonia distilled was determined spectrophotometrically by nesslerization at 625 mμ.

Qualitative method: One micro-mole of nicotinamide in 1 ml. of 0.05 M phosphate buffer was added to 1 ml. of extract, then placed in an incubator at 37°C. for three hours. Ammonia formed in the system was determined by adding 0.5 ml. of Nessler reagent directly without distillation of ammonia.

pH optimum of nicotinamidase: Buffer solutions

[1] From the Research Institute for Tuberculosis and Leprosy, Tohoku University, Sendai, Japan.

[2] The Aoyama B strain is used in Japan to make tuberculin.

[3] These strains are photochromogens and were obtained from Dr. Ernest H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah. They were cultured from sputum and from resected lung specimens.

[4] Guinea pig I and II strains were isolated from the viscera of guinea pigs in Japan.

for different hydrogen ion concentrations were prepared as follows: pH 5.0, sodium citrate and HCl buffer; pH 6.0 to 8.0, phosphate buffer; pH 9.0 to 10.0, borax NaOH buffer.

Effect of heat on nicotinamidase: Cell-free extracts of mycobacteria were heated in a water bath at 60°C. for fifteen minutes and at 100°C. for fifteen minutes. Nicotinamide was added and incubated, and the nicotinamidase activity was then determined by the above method.

Stability of nicotinamidase in frozen extracts: Cell-free extracts from mycobacteria were kept in a deep freeze of about −20°C. for two months and the nicotinamidase activity was then determined by the above method.

The asparaginase activity of various types of mycobacteria was determined by the same method as outlined above.

Results

Quantitative nicotinamidase activity of cell-free extracts of various types of mycobacteria (table 1 and figure 1): When 10 micro-moles of nicotinamide were added to the cell-free extracts from various types of mycobacteria, human tubercle bacilli (both the virulent and attenuated strains) produced 3.2 to 4.4 micro-moles of ammonia. No differences were observed between isoniazid-susceptible and isoniazid-resistant strains. The nicotinamidase activity of bovine tubercle bacilli was negligible: they produced an average of 0.12 micro-moles of ammonia irrespective of their virulence and

TABLE 1

NICOTINAMIDASE ACTIVITY OF EXTRACTS FROM VARIOUS MYCOBACTERIA
pH 7.0; Incubation at 37°C. for 3 Hours

Type	Strain	Substrate Nicotinamide	Ammonia Formed	Ammonia without Distillation	
				Nicotinamide	Nessler Reagent
		(*micro-moles*)	(*micro-moles*)	(*micro-moles*)	
Human tubercle bacilli	H37Rv	10	3.3	1	+
	H37Rv isoniazid-R	10	3.2	1	+
	H37Ra	10	3.7	1	+
	R1Rv	10	3.6	1	+
	Aoyama B	10	3.8	1	+
	H2	10	4.2	1	+
	JH16Ra	10	4.4	1	+
Bovine tubercle bacilli	Vallée	10	0.2	1	−
	Ravenel	10	0.1	1	−
	Ravenel isoniazid-R	10	0.1	1	−
	B-4228-3	10	0.2	1	−
	B-4228-4	10	0.1	1	−
	BCG-Japan	10	0.1	1	−
	BCG-Copenhagen	10	0.1	1	−
	BCG-Paris	10	0.1	1	−
Avian tubercle bacilli	4110	10	3.8	1	+
	4121	10	3.8	1	+
Unclassified mycobacteria	P-1	10	5.3	1	++
	P-8	10	3.3	1	+
	P-16	10	3.8	1	+
	P-22	10	3.2	1	+
Nonpathogenic acid-fast bacilli	*M. phlei*	10	6.8	1	++
	M. 607	10	6.8	1	++
	M. smegmatis	10	7.4	1	++
	M. ranae	10	6.2	1	++
	Guinea pig I	10	6.7	1	++
	Guinea pig II	10	6.5	1	++

− same as control
\+ yellow
++ orange

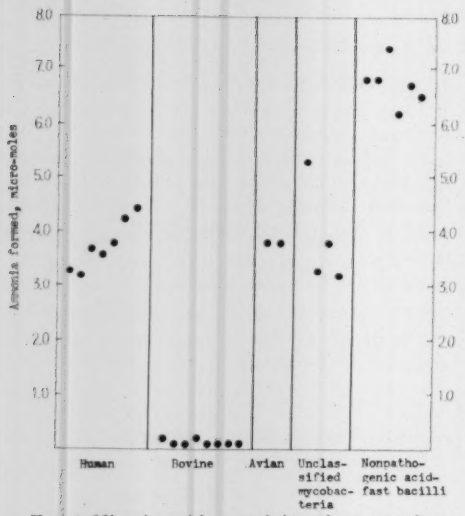

FIG. 1. Nicotinamidase activity of extracts from various mycobacteria; substrate nicotinamide, 10 micro-moles; incubation at 37°C. for three hours.

isoniazid susceptibility. Avian tubercle bacilli produced 3.8 micro-moles of ammonia. Unclassified mycobacteria produced an average of 3.9 micro-moles. Nonpathogenic acid-fast bacilli produced 6.7 micro-moles of ammonia. The great difference in nicotinamidase activity between bovine tubercle bacilli and other mycobacteria is shown in figure 1.

Qualitative nicotinamidase estimation: In performing the test qualitatively, the last procedure of distillation is omitted. After the incubation, Nessler reagent is added directly to the nicotinamide and cell-free extract mixture. Only bovine tubercle bacilli show no color change; human and avian bacilli and unclassified mycobacteria give a yellow color (+) reaction; and nonpathogenic acid-fast bacilli give an orange color (++) reaction. The bovine bacillus can thus be differentiated from the other mycobacteria by this qualitative color test. When Nessler's reagent is added to the mixture of nicotinamide and cell-free extracts directly, the reaction should be read immediately because, after a few minutes, a yellow color will appear in the control tube and make a comparison impossible. The cause of this yellow color change after a delay of a few minutes is not known. This phenomenon does not occur in the sample after distillation or Russel's phenol-hypochlorite method (8).

Asparaginase of cell-free extracts from mycobacteria: The asparaginase activity of H37Ra, P-8, and *M. 607* was determined for comparison with nicotinamidase (table 2). The asparaginase activity of all three strains tested showed similar values, with slightly higher activity in *M. 607*; no difference was observed between H37Ra and BCG. This is in contrast to the remarkable difference in nicotinamidase activity between bovine tubercle bacilli and other mycobacteria.

Influence of hydrogen ion concentration on nicotinamidase (figure 2): The nicotinamidase of human tubercle bacilli strain H37Ra, unclassified mycobacteria P-8 (photochromogenic mycobacteria), and saprophytic *M. 607* is active over a wide range of pH 6.0 to 9.0 with an optimal range of 7.0 to 8.0, as may be seen in figure 2. Very low nicotinamidase activity

TABLE 2

ASPARAGINASE ACTIVITY OF EXTRACTS FROM MYCOBACTERIA

pH 7.0; Incubation at 37°C. for 3 Hours

Strain	Substrate l-Asparagine	Ammonia Formed
	(*micro-moles*)	(*micro-moles*)
H37Ra	10	7.6
BCG	10	7.2
M. 607	10	8.2

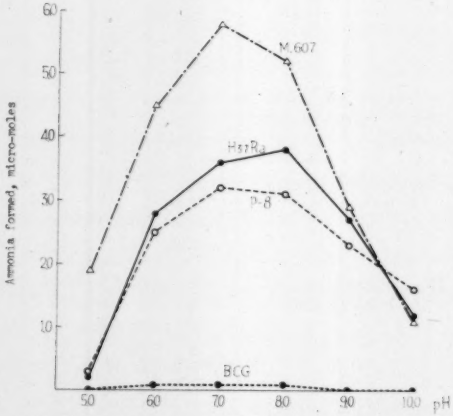

FIG. 2. Influence of pH on nicotinamidase of mycobacteria; substrate nicotinamide, 10 micro-moles; incubation at 37°C. for three hours.

TABLE 3

EFFECT OF HEAT ON THE NICOTINAMIDASE ACTIVITY OF MYCOBACTERIA

Substrate Nicotinamide, 10 Micro-moles; pH 7.0

Strain	Ammonia Formed		
	60°C. for 15 Minutes	100°C. for 15 Minutes	Control 37°C.
	(*micro-moles*)		
H37Ra	0.6 (16.2%)	0.1 (2.7%)	3.7 (100%)
M. 607	1.1 (16.1%)	0.2 (2.9%)	6.8 (100%)

TABLE 4

PRESERVATION OF NICOTINAMIDASE

−20°C. for 2 Months; Substrate Nicotinamide, 10 Micro-moles, Incubation at 37°C. for 3 Hours

Strain	Fresh Extract Ammonia	Two Months Preserved Ammonia
	(*micro-moles*)	
H37Ra	3.7 (100%)	2.6 (70.3%)
P-8	3.3 (100%)	2.8 (84.8%)

of BCG was observed over all of the pH ranges.

Effect of heat on nicotinamidase (table 3): As shown in table 3, the nicotinamidase activity of H37Ra, P-8, and *M. 607* was decreased more than 80 per cent by heating at 60°C. for fifteen minutes, and was almost completely lost by heating at 100°C. for fifteen minutes.

Stability of nicotinamidase in frozen extracts (table 4): Cell-free extracts which were kept for two months in deep freeze showed about 20 to 30 per cent decrease of enzyme activity as compared with the freshly prepared extract.

DISCUSSION

Bacterial amidases have received less attention than animal amidases. Nicotinamide is a component of pyridine nucleotides, coenzyme I and II, which play an important role in the oxidation-reduction of living cells. Some bacteria are able to synthesize nicotinamide from nicotinic acid. Only a few reports (5, 6) have appeared in the literature which show that mycobacteria produced the enzyme nicotinamidase which de-aminates nicotinamide to nicotinic acid.

The results of the present study show that nicotinamidases of mycobacteria are active over a wide pH range (between 6.0 and 9.0, the optimum being 7.0 to 8.0).

Heating at 60°C. for fifteen minutes destroys 80 per cent of the enzyme activity; complete destruction occurs at 100°C. Nicotinamidase can be preserved by freezing over a period of two months.

In the present study it was found, in general agreement with Bönicke and Lisboa (6), that bovine tubercle bacilli have very low nicotinamidase activity. This enzyme activity is not related to virulence or to isoniazid susceptibility, but is linked to the type of mycobacteria. The nicotinamidase activity of bovine tubercle bacilli is less than one-thirtieth of that of human tubercle bacilli, avian tubercle bacilli, or unclassified mycobacteria, and is less than one-sixtieth of that of nonpathogenic acid-fast bacilli. Since there have been no biochemical tests to distinguish bovine tubercle bacilli from other mycobacteria, this enzyme activity may serve this purpose.

In Europe, human infection by bovine tubercle bacilli is still a significant public health problem. The nicotinamidase test may be of assistance in solving individual cases.

Deaths following BCG vaccination have been reported in the literature. The fatal strains could not always be identified. This test may serve in establishing the type.

No differences were observed in the asparaginase activity of human and bovine tubercle bacilli and of nonpathogenic acid-fast bacilli, in contrast to the great difference in the nicotinamidase activity. This indicates that the low level of nicotinamidase activity in the bovine strains of *M. tuberculosis* is not a reflection of a general, but of a specific, low enzyme activity.

SUMMARY

The nicotinamidase activity of cell-free extracts from various mycobacteria was determined qualitatively and quantitatively.

Only bovine tubercle bacilli, irrespective of virulence and isoniazid susceptibility, reveal significantly lower nicotinamidase activity than other mycobacteria. The nicotinamidase activity of human, avian, and unclassified mycobacteria is more than thirty times that of bovine bacilli, while that of nonpathogenic acid-fast bacilli is

sixty times greater. Thus, bovine tubercle bacilli, both virulent and attenuated, irrespective of isoniazid resistance, can be distinguished from other mycobacteria by determining the nicotinamidase activity of cell-free extracts of the bacteria.

Sumario

Diferenciación de los Bacilos Tuberculosos Bovinos de Otras Micobacterias por la Determinación de la Actividad en Nicotinamidasa

Se determinó cualitativa y cuantitativamente la actividad nicotinamidásica de los extractos citoprivos de varias micobacterias.

Independientemente de la virulencia y la susceptibilidad a la isoniacida, sólo los bacilos tuberculosos bovinos revelan una actividad decididamente menor de la nicotinamidasa que las demás micobacterias. La actividad nicotinamidásica de las micobacterias humanas, aviarias e inclasificadas es más de treinta veces superior a la de los bacilos bovinos, mientras que la de los bacilos ácidorresistentes no patógenos es sesenta veces mayor. Haciendo caso omiso de su resistencia a la isoniacida, puede, pues, distinguirse a los bacilos tuberculosos bovinos, tanto virulentos como atenuados, de las otras micobacterias por la determinación de la actividad nicotinamidásica de los extractos citoprivos de las bacterias.

Resume

Moyen de différencier les bacilles tuberculeux bovins d'autres mycobactéries, par la détermination de l'activité de la nicotinamidase

L'activité de la nicotinamidase des extraits de "cellules libres" de différentes mycobactéries fut étudiée qualitativement et quantativement.

Il n'y a que les bacilles tuberculeux bovins, qui, sans tenir compte de leur virulence et de leur sensibilité à l'isoniazide, révèlent une activité nicotinamidasique notablement plus faible que les autres mycobactéries. L'activité nicotinamidasique des mycobactéries humaines, aviaires et non classées est trente fois supérieure à celle des bacilles bovins, tandis que celle des bacilles acidorésistant non pathogène est soixante fois plus grande. Ainsi, les bacilles bovins tuberculeux, virulents et atténués, sans considérer leur résistance vis à vis de l'isoniazide, peuvent être distingués des autres mycobactéries par l'appréciation de l'activité nicotinamidasique d'extraits de "cellules libres" de ces bactéries.

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THE ACTION OF CHLORPROMAZINE UPON MYCOBACTERIUM TUBERCULOSIS[1,2]

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INTRODUCTION

The rationale for this study was based upon two reports suggesting that drugs active upon certain enzymes vital to the tubercle bacillus might be able to inhibit this organism selectively *in vivo*. The points involved were: first, that mycobacteria among other organisms (1) produce esterases acting upon short-chain aliphatic lipids, i.e., ali-esterases (2, 3); and second, that these ubiquitous enzymes are of great importance to bacteria but are not essential to higher organisms (3).

If an enzyme inhibitor is to be useful therapeutically as an antimicrobial agent, it should be active upon the organism while sparing the more highly organized host. Mendel and associates (3) demonstrated that the rat is unharmed by suppression of ali-esterases, employing an inhibitor which was not toxic for this animal. Although this inhibitor (tri-o-cresyl phosphate-TOCP) had a marked effect upon ali-esterases in serum, liver, and other tissues of this animal, its action was too slow to keep pace with the formation of esterase in such rapidly multiplying cells as microorganisms. Consequently, it was not considered to be suitable as a potential therapeutic agent. Ruys (4) worked with a more potent enzyme inhibitor, an alkyl phosphate derivative, diethyl - *p* - nitrophenol - phosphate (E660). This inhibited acid-fast bacilli very well in the test tube, but was too toxic for parenteral administration to animals.

A possibly useful lead in the desired direction was supplied in a report by Bernsohn and coworkers (5) who studied the action of chlorpromazine (*see* figure 2) upon brain enzyme systems. Ali-esterase of the brain was completely inhibited by chlorpromazine, while serum ali-esterase was inhibited to the extent of 50 per cent. On the basis of this finding, it was thought that this widely used drug should be investigated for its antimycobacterial effect *in vitro* and *in vivo*, and eventually in patients, as reported in the accompanying paper (6).

In addition to chlorpromazine, a number of related phenothiazine derivatives and other esterase inhibitors have been tested for their activities against *Mycobacterium tuberculosis*.

METHODS

The drugs used were chlorpromazine, an isomer of chlorpromazine, promazine, promethazine, pyrathiazine, diethazine, mepazine, and prochlorperazine (figure 1). Chlorpromazine sulfoxide was tested as a major urinary excretion product of chlorpromazine, and one drug with anticholinesterase action, physostigmine, was also included in the tests.

All substances were dissolved in physiologic saline for addition to the medium used for testing antibacterial properties except mepazine which, because it precipitated in saline, was dissolved in distilled water.

The test medium was Dubos liquid albumin containing Tween®. The Tween was included to keep growth as dispersed as possible for photometric readings, but the question arose whether it might influence the activities of the drugs. In one experiment this point was tested with chlorpromazine and mepazine; simultaneous tests with and without Tween revealed no differences in results.

Bacteriostatic tests were set up as follows: Duplicate tubes containing 5.3 ml. of medium were inoculated with desired concentrations of each drug in 0.6 ml. of saline (or distilled water in the case of mepazine). To all tubes, including controls without drug but with the same quantity of diluting fluid, 0.1 ml. of a suspension of *Mycobacterium tuberculosis* var. *hominis* was added. This culture was seven to ten days of age, grown in Dubos-Tween medium, and diluted with the same medium to a Klett reading of 20 (green filter). In some experiments the virulent strain H37Rv was employed; in others, the avirulent derivative H37Ra. Comparative tests with a number of the better-acting drugs indicated that there was no difference in activity upon these two strains. The

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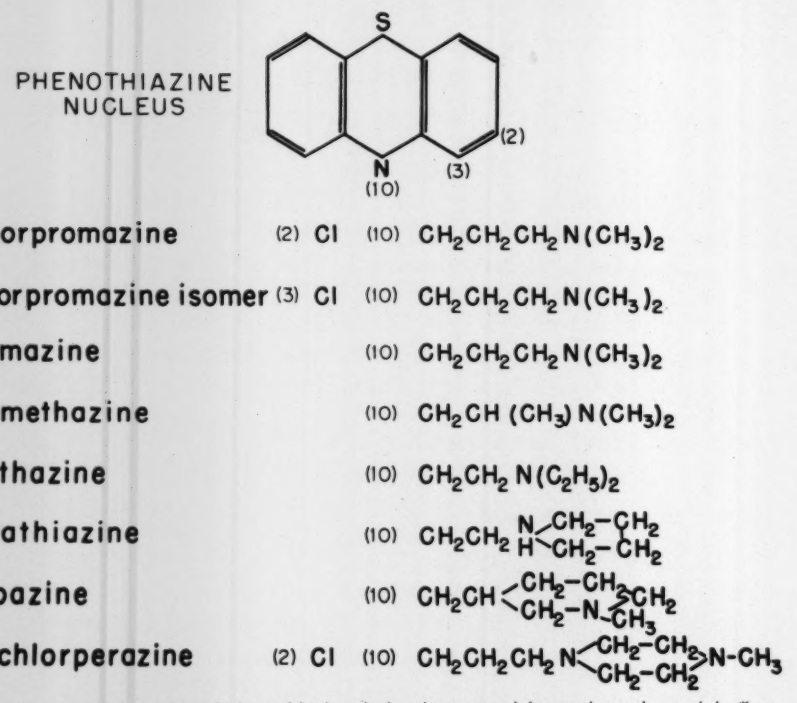

FIG. 1. Structural formulas of phenothiazine derivatives tested for antimycobacterial effect.

progress of bacillary growth was followed by daily Klett readings for periods up to twenty-one days.

It was desirable to learn whether certain of the more active drugs had bactericidal as well as bacteriostatic effects. Subcultures were made from the tubes on the day of inoculation to establish a baseline of colony counts, and this procedure was repeated usually on the seventh and twenty-first days of incubation. Aliquots of the shaken tubes were appropriately diluted in Tween-albumin medium, and 0.1 ml. of each dilution from each tube was spread over the surface of three Petri dishes of 5-cm. diameter containing Dubos oleic-acid–albumin agar. These plates were sealed and incubated for four weeks before final colony counts were made.

Tests for the production of ali-esterases by tubercle bacilli, and for the possible inhibitory effect upon these of certain of the drugs tested, were carried out by the modified method of Goldstein and associates (7). Two and one-half milliliters of tributyrin were emulsified in a mixture of 100 ml. of 0.5 per cent sodium diethyl barbiturate and 100 ml. of 2 per cent calcium acetate. Emulsification was accomplished in a Waring Blendor. For carrying out tests with tubercle bacilli, about 100 mg. wet weight of tubercle bacilli were washed once in 5 per cent glucose in saline solution and resuspended in 5 ml. of this same solution. The total suspension was added to 20 ml. of the emulsified substrate in a 500-ml. Erlenmeyer flask. The substrate had been prewarmed for about five minutes in a 37°C. water bath. For each assay a blank without bacilli was used, as well as a control containing all ingredients of the test, but was immediately inactivated after mixing by the addition of 100 ml. of ether-ethanol mixture, 1:9. The test flask and blank were incubated for two hours at 37°C. with occasional shaking. At the end of this time, all three flasks were inactivated by adding the ether-ethanol mixture described. The flask contents were then titrated to an endpoint at pH 10.65 by additions of increments of 0.05 N potassium hydroxide, with electrometric determination of the pH after each addition. The difference between the quantity of potassium hydroxide necessary to reach this endpoint in the test and that in the controls is equivalent to the amount of 0.05 N acid released by the hydrolysis of tributyrin.

RESULTS

The inhibitory properties of chlorpromazine and other phenothiazine derivatives in vitro: The results of all bacteriostatic experiments are shown in figure 2. In eight experiments, chlorpromazine

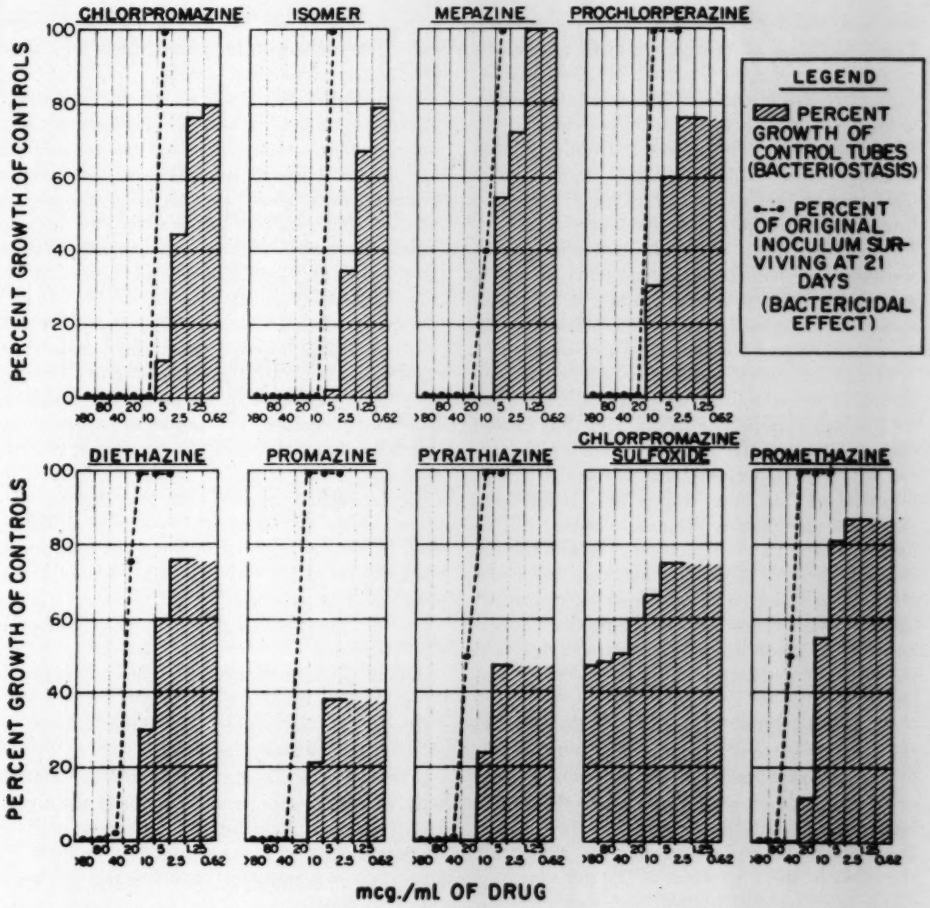

FIG. 2. Antimycobacterial activity of drugs expressed as per cent of growth in control tubes without drugs.

was set up in concentrations ranging from 80 through 0.62 γ per ml., by serial dilutions. These tests consistently showed complete suppression of bacillary growth through 10 γ per ml., about 90 per cent inhibition at 2.5 γ, and a small degree of inhibition, about 20 per cent, at the lowest concentration used. The proprietary vehicle in which the chlorpromazine was dissolved (containing ascorbic acid, sodium sulfite, sodium bisulfite, and sodium chloride) had no effect whatever when employed in the same concentrations.

An isomer of chlorpromazine was tested in the same series of dilutions in four experiments and showed a somewhat more pronounced effect; 5 γ in three of the experiments were found to be completely inhibitory to growth.

Mepazine was tested in six experiments, and like chlorpromazine showed good activity through 10 γ per ml. concentration. However, below this point its effectiveness diminished rapidly. The rest of the phenothiazine drugs, including prochlorperazine, promazine, pyrathiazine, promethazine, and diethazine, were less active (figure 2).

Chlorpromazine sulfoxide,[3] a urinary excretion product of chlorpromazine (8, 9), contrasted markedly in its activity with chlorpromazine it-

[3] Kindly supplied by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

self and the other phenothiazine compounds. Total inhibition of bacillary growth required a concentration of 2,500 γ per ml. of the substance, while with 310 γ, the next highest concentration used, the inhibition was only 53 per cent.

To test the susceptibilities of freshly isolated strains, similar liquid medium bacteriostatic tests were set up with cultures of bacilli recovered from the sputum of 2 patients. The second subcultures of these in Dubos-albumin-glucose medium were tested for susceptibility to chlorpromazine. Both showed inhibition in the same range of concentrations as were exhibited by the laboratory strains employed in this work.

The anticholinesterase physostigmine has been described as inhibiting ali-esterases also when used in high concentrations (3). This drug caused color changes in the liquid medium which precluded direct determinations of bacillary suppression, so that plate counts were made from the tubes immediately after inoculation, and on days 1, 7, and 21 of incubation. The two highest concentrations of physostigmine used, 300 γ and 150 γ, both appeared to have some bacteriostatic effect during the first week of incubation as compared with lower doses and control tubes, but by the end of twenty-one days this effect was not observable.

Bactericidal effects of drugs: In order to assess the possible bactericidal properties of these various drugs, quantitative enumerations of colonies on plates prepared after various periods of incubation of bacilli in the presence of the drugs in fluid medium were carried out. The results are shown in figure 2 in terms of the percentage of viable units of the original inoculum surviving after twenty-one days of incubation. All eight of the phenothiazine compounds were bactericidal through 80 γ per ml. of medium. In fact, this was found to be the case after one week of incubation, although these results are not shown. At 40 γ, however, differences become apparent; chlorpromazine, the chlorpromazine isomer, mepazine, and prochlorperazine were completely bactericidal, while the others were less active. At 20 γ, the chlorpromazine isomer was the only drug having complete bactericidal activity, while chlorpromazine, prochlorperazine, and mepazine showed almost complete activity. At 10 γ, only chlorpromazine and the chlorpromazine isomer of the eight drugs tested revealed almost complete bactericidal activity. Thus, the two drugs (chlorpromazine and its isomer) which were most striking in bacteriostatic activity were also most effective as bactericidal agents.

The anti-ali-esterase activity of chlorpromazine: The antimycobacterial activity of chlorpromazine and the other compounds tested can be ascribed only inferentially to inhibition of ali-esterases without some evidence that these substances can interfere with such enzymes produced by tubercle bacilli. As noted before, several investigators have found that ali-esterases are produced by these bacteria (1, 2, 10, 11).

Experiments were set up with cultures of BCG grown in Dubos liquid medium and harvested at one week. About 125 mg. moist weight of bacilli were used per test with tributyrin as substrate, as described earlier. For purposes of inhibiting esterase activity, 500 γ of chlorpromazine per ml. were used.

Representative experiments provided the following order of results, the quantity of potassium hydroxide required to bring the mixture to the endpoint at pH 10.65 being a measure of the activity of ali-esterase:

Bacilli plus substrate—9.6 ml. potassium hydroxide

Bacilli plus substrate plus chlorpromazine—5.6 ml. potassium hydroxide

Bacilli plus substrate, inactivated (control)—3.0 ml. potassium hydroxide

These findings suggest that chlorpromazine may inhibit the ali-esterase activity of the organisms, although some other deleterious activity of the drug upon the bacilli could account for this result as a secondary manifestation of bacillary inhibition.

Experiments in vivo: On the basis of the bacillary inhibition seen *in vitro*, chlorpromazine was tested for its possible therapeutic effects in mice and guinea pigs.

In two experiments in which adult mice (25 to 30 gm.) were used, the drug was injected subcutaneously in the amount of 20 mg. per kg. (The LD_{50} after subcutaneous injection in mice is stated to be 160 to 200 mg. per kg. (12).) Injections were begun on the day of, or ten days after, the initiation of infection, and in both cases administration was continued on a six-day-a-week basis.

This dose of drug was found to be toxic as evidenced by poor appearance, loss of weight, and the death of a number of the treated animals by the fifteenth day after infection. Examinations

of the lungs for lesions and acid-fast bacilli were carried out in paired animals, i.e., drug-treated and untreated infected controls, at times dictated by the death or sacrifice of the treated animals. No observable benefits were conferred by the drug.

Two similar experiments were carried out in guinea pigs. Again the drug was given beginning either on the day of infection or at a later time, in this case on day 28 of infection. In one experiment chlorpromazine was injected intramuscularly daily, in a dose of 17 mg. per kg. This occasioned considerable necrosis of tissue and loss of weight. In the second experiment the same dose of drug was given daily by gavage. In this case weight loss did not occur beyond that seen in infected control animals. In both experiments, pathologic examination of sacrificed subjects at seven weeks after the initiation of infection showed no differences between infected animals, both treated and control.

These results coincide with those reported by Nishimura and associates (13), and in themselves afforded no promise that chlorpromazine might be effective in the treatment of human tuberculosis, except perhaps as an adjunct drug along with the commonly employed antimicrobial agents. This possibility was not tested in animals, although the above-named workers failed to show an influence of combined administration of chlorpromazine and isoniazid in tuberculous mice.

Discussion

The present investigation of the antimycobacterial activities of chlorpromazine and related phenothiazine compounds was undertaken on the thesis that these substances may interfere with enzymes vital to the bacteria, but of relative unimportance to potential mammalian hosts. Anti-infectious effects of phenothiazine have been known for some time, aside from the present theoretic considerations. Phenothiazine was studied by De Eds and associates (14, 16), with special reference to infections of the urinary tract, in which it was effective (though soon replaced by sulfonamides), and also in helminthic infestations (16) in which, in some instances, it was also efficacious (17).

More recently, chlorpromazine itself has been tested clinically and in laboratory studies as an anti-infectious agent. Thus Geiger and Finkelstein (18) had occasion to treat one tuberculous schizophrenic patient with the drug, and found an unlooked-for improvement in the roentgenographic appearance of his lung sufficiently striking that they investigated the bacteriostatic action of the drug *in vitro*. It was found to be active in concentrations greater than 5 γ per 100 ml. Hayes (19) reported a similar experience with one patient. Nishimura and associates (13) found chlorpromazine especially effective against *M. tuberculosis* but rather weakly active upon *Staphylococcus aureus*. It was, however, thought to have a synergistic action in the test tube when combined with various antimicrobials; this effect was apparent to some extent against tubercle bacilli, but more strikingly with other organisms. Chlorpromazine was tested for its therapeutic abilities in tuberculous mice alone and combined with isoniazid, but no beneficial effects were observed.

Contrary observations have also been described. Maral and Cosar (20) found that chlorpromazine enhanced infections by pneumococci in mice, and Grosz and Norton (21) reported an untoward effect in *Salmonella enteriditis* infections in these animals. At least one clinical report suggests also that infections may frequently complicate the institutional use of chlorpromazine as well as reserpine (22). It has been said that the drug may have a cortisone-like action in impairing the function of reticuloendothelial cells (23) and depressing the phagocytic activities of leukocytes (24).

The influence of the drug in experimental viral infections has also been assayed. Maral (25) found no real effect of the drug upon the courses of influenza or encephalomyelitic viral infections in mice. Pearson and co-workers (26) found chlorpromazine to be inhibitory for Theiler's GD7 virus in minced mouse-brain suspension, but the drug did not protect intracerebrally infected mice from death.

The present studies in general corroborate other experiences with chlorpromazine as an antimycobacterial agent in the test tube, and extend this activity to other phenothiazine derivatives. This activity is predicated upon an inhibitory influence of these compounds upon lipases (ali-esterases) produced by the bacteria and essential to their metabolic integrity. Experimental evidence in part corroborates this viewpoint in that chlorpromazine interferes markedly with the ability of tubercle bacilli to

attack a tributyrin substrate. It is possible that the antibacterial effectiveness of these compounds is not a result of this anti-enzyme effect; cause and effect may be reversed; an antibacterial influence of unknown character may result in the failure of the bacterium to produce the enzyme.

The concentrations at which these drugs are effective *in vitro*, and their relative nontoxicity in human beings and animals as attested by their widespread use for other purposes, led the present writers to preliminary experiments concerned with therapeutic efficacy *in vivo*. Studies in mice and guinea pigs were not rewarding in this respect, in agreement with the work of Nishimura and others (13), which only recently came to the attention of the present writers.

The phenothiazine compounds are of much interest now in neuropharmacologic connotations, so that many derivatives are being prepared and tested for toxicity. It may be worth while to take advantage of this situation to the extent of assaying these compounds for their potential chemotherapeutic properties in infectious states.

Summary

Chlorpromazine is bacteriostatic for *M. tuberculosis* in concentrations of 5 γ per ml. It is bactericidal in minimal concentrations of 10 γ per ml. An isomer of chlorpromazine has somewhat better static properties. A variety of other phenothiazine derivatives are antimycobacterial also, in lesser degree.

The urinary excretion product of chlorpromazine, chlorpromazine sulfoxide, has very little effect upon the bacillus.

Chlorpromazine inhibits to a large extent the ali-esterase activity of tubercle bacilli; its antibacterial action may depend upon this fact. This drug was not therapeutically effective in tuberculous infections in mice or guinea pigs.

Sumario

La Acción de la Clorpromacina sobre el Mycobacterium Tuberculosis

La clorpromacina es bacteriostática para el *M. tuberculosis* a concentraciones de 5 γ por ml. Es bactericida a concentraciones mínimas de 10 γ por ml. Un isómero de la clorpromacina posee propiedades estáticas algo mejores. Varios otros derivados de la fenotiacina son también antimicobacterianos en menor proporción.

El producto de la excreción urinaria de la clorpromacina, el sulfóxido de clorpromacina, ejerce muy poco efecto sobre el bacilo.

La clorpromacina inhibe en gran proporción la actividad aliestereásica del bacilo tuberculoso; su acción antibacteriana tal vez dependa de esta propiedad. Esta droga no resultó terapéuticamente eficaz en las infecciones tuberculosas en los ratones o los cobayos.

Resume

Action de la chlorpromazine sur le Mycobacterium tuberculosis

La chlorpromazine est bactériostatique pour le *M. tuberculosis* à la concentration de 5 γ par ml. Elle est bactéricide à la concentration minima de 10 γ par ml. Un isomère de la chlorpromazine présente des propriétés bactériostátiques quelque peu supérieures. Une quantité d'autres dérivés de la phénothiazine sont antimycobactériens aussi mais à un degré moindre.

Les produits d'excrétion de la chlorpromazine dans l'urine, le sulfoxide de chlorpromazine, a trés peu d'action sur le bacille.

La chlorpromazine inhibe dans une large mesure l'activité aliestérasique du bacille tuberculeux. Son action antibactérienne dépend peut-être de ce facteur. Ce médicament n'a pas été efficace au point de vue thérapeutique dans l'infection tuberculeuse de la souris et du cobaye.

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CHLORPROMAZINE IN NONPSYCHOTIC PATIENTS WITH PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

Beneficial effects from chlorpromazine in treating pulmonary tuberculosis have been attributed to the sedative action of the drug, either alone (1) or combined with other sedatives to produce "sleep therapy" (2). Other workers have described anti-inflammatory properties which, when combined with conventional antimicrobials, have been particularly effective against exudative disease (3). Finally, it has been suggested, on the basis of *in vitro* experiments, that chlorpromazine has antimicrobial effects of its own (1, 4).

The laboratory studies described in the preceding paper (5) were the basis for the clinical trial reported here. The premise was that chlorpromazine might be effective in patients with pulmonary tuberculosis because of its demonstrated bacteriostatic and bactericidal effects against *Mycobacterium tuberculosis*. In tuberculous psychotic patients receiving the drug for psychosis, spectacular improvement of the pulmonary disease has been noted occasionally (6, 7).

METHOD OF STUDY

Ideally, it is preferable to study only one drug at a time in any disease process. Such a study was not considered feasible for the present group of patients. All patients available for this study were already being treated with conventional antimicrobials, and it seemed unwise to replace a known effective treatment with one of unproved merit. Accordingly, it was decided to evaluate chlorpromazine in pulmonary tuberculosis by adding the drug to established treatment programs.

Patients selected for this study were characteristic of those admitted to a public tuberculosis hospital. Almost all were chronic cases, most had sputum positive for *M. tuberculosis*, and many had not shown adequate response to a long trial of antimicrobials. The economic and social status of these patients was usually low. Many had previously shown some evidence of psychopathology in the form of alcohol addiction, poor work records, or poor cooperation in previous treatment programs.

Most of the patients had been hospitalized long enough so that many prior examinations of sputum and chest roentgenograms were available. Thus the clinical course could be well assessed. Before entering the study, two successive sputum smears and a new chest roentgenogram were obtained. During the period of study, several smears and cultures of sputum were obtained, and chest roentgenograms were repeated at three-month intervals. The chest films were read and the sputum studies interpreted by persons unaware of the specific treatment given individual patients. Chest films were read as improved only if definite changes were observed following a period of recent stability. Sputum was considered as showing equivocal changes if the frequency of infectious specimens declined and the last three sputum specimens were negative for tubercle bacilli. Sputum "conversion" was considered to be attained if successive examinations were negative for six months or more. Records of daily temperature and monthly weight were kept on each patient. No other special laboratory tests were performed unless clinically indicated.

Originally it was planned to use a double-blind technique in this study. Twenty-three patients received 300 mg. of chlorpromazine daily and 34 received inactive placebos for a period of three months. Despite the fact that initiation of treatment with small doses gradually increased to the 300 mg. level, pharmacologic effects of the drug disclosed its presence in about three-quarters of the patients. Instead of the double-blind control, it was decided to use a comparison group of patients similar to those treated with chlorpromazine.

These patients came from the initial placebo group, from those who had had brief courses of the drug but who had asked to be taken off, and from patients later nominated for the study but never given chlorpromazine. Ultimately, 36 patients were treated with 300-mg. doses of chlorpromazine daily. Twelve received the drug from three to five months, 7 from six to eight months, and 17 from nine to twelve months. A comparison group of 37 patients was observed for similar periods without the addition of chlorpromazine to their therapeutic regimens.

The clinical characteristics of the chlorpromazine-treated and the comparison groups are summarized in table 1. In general the two groups were quite similar. However, the comparison

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TABLE 1

CLINICAL CHARACTERISTICS OF THE CHLORPROMAZINE-TREATED GROUP AND THE COMPARISON GROUP AT BEGINNING OF STUDY

Characteristic	Chlorpromazine-treated Group 36 Cases	Comparison Group 37 Cases
Age		
30–39 years	4	0
40–49 years	10	8
50–59 years	14	12
More than 60 years	8	17
Stage of tuberculosis		
Minimal	1	2
Moderately advanced	13	13
Far advanced	22	22
Bacterial diagnosis		
Less than 1 year	9	5
1–3 years	11	8
More than 3 years	13	24
Collapse therapy		
Air	17	17
Surgical	3	2
Both	3	1
Total	23	20
Concomitant antimicrobials		
Isoniazid–PAS	4	1
Isoniazid–streptomycin	4	7
Isoniazid–streptomycin–PAS	28	29
Duration of antimicrobial therapy		
Less than 6 months	7	4
6–12 months	7	8
More than 12 months	22	25
Roentgenographic findings		
Cavitation present	17	23
Lesion stable for 6 months or more	24	23

group tended to include older patients with longer known disease and more roentgenographic evidence of cavitation. To some extent these factors might have tended to bias results in favor of the chlorpromazine-treated group.

RESULTS OF STUDY

The results of adding 300 mg. of chlorpromazine daily to a program of antimicrobial treatment of pulmonary tuberculosis are summarized in table 2. The difference in reversal of infectiousness between the chlorpromazine-treated patients and the comparison group was not statistically significant. However, when equivocal sputum changes were grouped with sputum "conversions" as representing improvement, the difference between the treated and comparison groups was significant.

Roentgenographic improvement was uncommon in both groups, with no significant difference between them. Because of the rigid criteria used for roentgenographic improvement and the chronic far advanced disease of so many patients, the likelihood of detecting definite changes was reduced.

To eliminate the possibility that inadequate duration of treatment might have masked possible therapeutic effects, those patients treated with chlorpromazine for nine to twelve months were considered as a special group. When 17 such patients were compared with 20 similar patients in the comparison group (table 3), the differences in reversal of infectiousness and roentgenographic improvement were not statistically significant. Thus, longer treatment with chlorpromazine did not enhance the results obtained.

Changes in sputum did not correlate well with roentgenographic improvement. Only one of the 7 patients in the chlorpromazine-treated group with reversal of infectiousness showed roentgenographic improvement. The remaining 6 sputum "converters" had no evident improvement in their chest roentgenograms. In the comparison group, only one of 3 "converters" also had roentgenographic improvement; the remaining 2 had no change. Roentgenographic improvement was observed in 4 chlorpromazine-treated

TABLE 2

TREATMENT OF PULMONARY TUBERCULOSIS WITH 300 MG. OF CHLORPROMAZINE DAILY AS COMPARED WITH UNTREATED GROUP

Chlorpromazine-treated group—36 patients
- Sputum studies—29 patients with initially infectious sputum
  - 17 remained infectious
  - 5 showed equivocal changes
  - 7 became noninfectious
- Roentgenographic studies
  - 29 unchanged
  - 7 improved

Comparison group—37 patients
- Sputum studies—34 with initially infectious sputum
  - 29 remained infectious
  - 2 showed equivocal changes
  - 3 became noninfectious
- Roentgenographic studies
  - 34 unchanged
  - 3 improved

TABLE 3

TREATMENT WITH CHLORPROMAZINE (9 TO 12 MONTHS) COMPARED WITH UNTREATED GROUP OBSERVED FOR SIMILAR PERIOD

Chlorpromazine-treated group—17 patients	
Sputum studies—17 with initially infectious sputum	
12 remained infectious	
2 showed equivocal changes	
3 became noninfectious	
Roentgenographic studies	
14 unchanged	
3 improved	
Comparison group—20 patients	
Sputum studies—20 with initially infectious sputum	
16 remained infectious	
1 showed equivocal changes	
3 became noninfectious	
Roentgenographic studies	
19 unchanged	
1 improved	

patients whose sputum changes were equivocal and in one whose sputum had not been positive for *M. tuberculosis*. One chlorpromazine-treated patient and 2 in the comparison group had roentgenographic improvement without any change in sputum.

Because reversal of infectiousness and roentgenographic improvement correlated poorly, the total of patients showing either evidence of improvement was larger than the number showing each change alone. Thus, 13 of the 36 chlorpromazine-treated patients manifested improvement either on chest films or by reversal of infectiousness, while 5 of the 37 in the comparison group had similar improvement. These differences in the total number of patients showing improvement are statistically significant in favor of the chlorpromazine-treated group at the 95 per cent level of confidence.

To determine if drug-resistant strains of tubercle bacilli appeared during treatment, sputum specimens were obtained from 4 patients treated with chlorpromazine for more than nine months and from 2 untreated patients. Subcultures of the isolated organisms were tested against 20, 10, and 5 γ per ml. concentrations with readings at thirteen and twenty days. Organisms from 2 patients not treated with the drug were inhibited in the same range as those obtained with laboratory strains. So were organisms from 2 patients treated with chlorpromazine. One strain from a treated patient showed some increased resistance in the presence of 5 and 10 γ read at twenty days. The strain from the fourth patient showed a definite increase of resistance, being inhibited only at the 20 γ per ml. level; this organism had also developed resistance to isoniazid. Although none of these patients had reversal of infectiousness, one (whose organism remained susceptible) had roentgenographic evidence of improvement.

Weight gain was appreciable during chlorpromazine therapy. Twenty of the 36 chlorpromazine-treated patients gained more than 5 pounds in weight, with gains up to 25 pounds. Only 3 chlorpromazine-treated patients lost more than 5 pounds during treatment. Seven of the 37 patients in the comparison group gained more than 5 pounds, while 5 lost more than that amount. The average weight gain for the entire treated group amounted to 7 pounds; for the comparison group, only 1 pound.

Minor side reactions were common in the treated group and rare in the comparison group. Especially noteworthy among chlorpromazine-treated patients were lethargy, somnolence, dizziness, nasal congestion, and dry mucous membranes. Some patients had drier sputum with increased difficulty in expulsion, a factor likely to influence the results of sputum studies.

The major complications encountered in the treated group are listed in table 4. Of particular interest was the frequency of adverse mental symptoms in this "normal" group. Four patients developed transient psychotic episodes, two manifested by depression, one with psychomotor agitation, and one with a mixture of depression and agitation. Symptoms appeared within three weeks to four and one-half months of treatment with the drug; but in 2 patients, a prior course of placebo for three months was without any adverse effect. In 3 of these patients, there was obvious evidence of pre-existing psychopathology

TABLE 4

MAJOR COMPLICATIONS OF CHLORPROMAZINE TREATMENT IN 36 TUBERCULOUS PATIENTS

Manifestation	Number Affected
Syncope	4
Extrapyramidal syndrome	4
Jaundice	2
Fever	1
Psychosis	4
Nervousness	6
Withdrawal symptoms	4

(alcoholism, schizoid personality). Two patients required transfer to psychiatric units for the management of their psychoses. Six other patients experienced increased nervousness, insomnia, and a vague feeling of apprehension while on the drug. At the termination of the drug trials, chlorpromazine was stopped rather abruptly. Four patients complained of increasing nervousness, restlessness, insomnia, and gastrointestinal symptoms. Resuming the drug or substituting other sedatives managed these mild withdrawal reactions easily.

Discussion

These studies do not justify any firm conclusion about the efficacy of chlorpromazine in pulmonary tuberculosis. Statistically significant differences in favor of the chlorpromazine-treated group were obtained in regard to the decreased infectiousness of sputum and to the total number of patients improved. Nevertheless, the evidence was far from conclusive. It is disturbing that similar differences were not noted in the group treated for the longest period (nine to twelve months).

Of the nonspecific effects of chlorpromazine on pulmonary tuberculosis, only that of weight gain was worth considering. Similar weight gain has been noted frequently in psychiatric patients treated with the drug (8). Whether increased sedation in nondisturbed patients has any ameliorating effect on the disease process is questionable. In the patients of the present study there was some indication that chlorpromazine increased patient cooperation, an indirectly beneficial effect.

Only the adverse mental reactions were unexpected complications of chlorpromazine treatment. The precipitation of actual psychoses and the exacerbation of anxiety reactions were surprising, both in frequency and in severity. The possibility that concurrent administration of isoniazid might have increased such reactions is raised by a report of confusion and other mental symptoms in 5 patients treated with chlorpromazine and isoniazid concurrently (9). The withdrawal reactions were mild, probably because the drug has a relatively long span of pharmacologic effect. Recently, similar withdrawal reactions were reported in psychiatric patients treated with chlorpromazine for long periods (eighteen to forty-four months) (10).

Despite the unconvincing therapeutic effects from chlorpromazine in patients with pulmonary tuberculosis, other phenothiazine derivatives should be screened for use in such patients. Many derivatives with various degrees of pharmacologic action are being developed. Some may prove to be clinically applicable to the treatment of tuberculosis.

Summary

Thirty-six patients with pulmonary tuberculosis were treated by adding chlorpromazine to the customary antimicrobial treatment. Daily doses of 300 mg. were given for periods of three to twelve months, the majority of patients being treated for more than six months. Progress was evaluated by microscopy and culture of sputum and serial roentgenograms of the chest. Results were compared with those of a similar group of 37 patients who did not receive chlorpromazine but who were evaluated similarly and concurrently.

Total evidence of improvement (either by roentgenographic criteria or reversal of infectiousness) in the chlorpromazine-treated group was significantly greater than in the comparison group. Decreased infectiousness of sputum was also significantly different in the treated group. Neither reversal of infectiousness nor roentgenographic improvement considered separately was significantly different in the entire treated group or in those observed for the longest period (from nine to twelve months).

Weight gain was greater in the treated group. Minor and major side reactions of the usual type were experienced from chlorpromazine treatment. Adverse mental symptoms, such as precipitation of psychosis, exacerbation of anxiety reactions, and mild withdrawal reactions, were unexpected complications.

At present, chlorpromazine cannot be recommended as an adjunctive treatment for the nonpsychotic patient with pulmonary tuberculosis, despite its demonstrated *in vitro* antimicrobial activity. However, other related compounds may appear with more antimicrobial activity and less toxic effects.

Sumario

La Clorpromacina en los Enfermos de Tuberculosis Pulmonar sin Psicosis

A 36 tuberculosos pulmonares se les trató agregando clorpromacina al régimen antimicrobiano de costumbre. Se administraron dosis diarias de 300 mgs. durante períodos de tres a

doce meses, tratándose por más de seis meses a la mayoría de los enfermos. El adelanto fué justipreciado por la microscopía y el cultivo del esputo y las radiografías seriadas del tórax. Se compararon los resultados con los observados en un grupo semejante de 37 enfermos que no recibieron clorpromacina, pero que fueron justipreciados en forma similar y concurrentemente.

Los signos totales de mejoría (ya por pautas radiográficas o viraje de la infecciosidad) en el grupo tratado con clorpromacina fueron decididamente mayores que en el grupo de comparación. La disminución de la infecciosidad del esputo fué también notablemente distinta en el grupo tratado. Considerados por separado, ni el viraje de la infecciosidad ni la mejoría roentgenográfica fueron mayormente distintos en todo el grupo tratado o en los enfermos observados durante el período más prolongado (de nueve a doce meses).

El aumento de peso fué mayor en el grupo tratado. Hubo reacciones colaterales mayores y menores de la forma habitual al tratamiento con clorpromacina. Complicaciones inesperadas fueron los síntomas mentales adversos, tales como la precipitación de psicosis, la exacerbación de reacciones de ansiedad y las leves reacciones de supresión.

Por ahora, a pesar de su demostrada actividad antimicrobiana *in vitro*, no cabe recomendar la clorpromacina como tratamiento coadyuvante en el tuberculoso pulmonar no psicótico. Sin embargo, tal vez aparezcan otros compuestos afines que posean mayor actividad antimicrobiana y menos efectos tóxicos.

Resume

La chlorpromazine chez des malades non psychotiques ayant une tuberculose pulmonaire

Trente six malades ayant une tuberculose pulmonaire furent traités en ajoutant de la chlorpromazine au traitement habituel antimicrobien. Des doses journalières de 300 mg furent données pendant des périodes de trois à douze mois, la majorité des malades étant traités pendant plus de six mois. Les progrés furent jugés d'après la culture et l'examen au microscope de l'expectoration et des radiographies en séries des poumons. Les résultats furent comparés avec ceux obtenus chez un groupe de 37 malades semblables qui ne reçurent pas de chlorpromazine mais qui furent examinés d'une facon semblable et en même temps.

Une complète evidence d'amélioration (soit d'aprés les examens radiographiques soit par la négativité de l'expectoration) dans le groupe traité par la chlorpromázine fut nettement plus grande que dans le groupe comparé. Une expectoration moins positive fut aussi un changement net dans le groupe traité. Ni la négativité de l'expectoration, ni l'amélioration des images radiographiques considérées séparément, ne furent réellement changés dans tout le groupe traité et chez les malades observés le plus longtemps (de neuf à douze mois).

L'augmentation de poids fut plus grande dans le groupe traité. Des réactions mineures et importantes, du type habituel, furent notées dans le traitement à la chlorpromazine. Des symptômes mentaux, tels que le déclenchement de psychoses, des bouffées de réactions anxieuses et de légères réactions à l'arrêt de la chlorpromazine furent des complications inattendues.

A présent, la chlorpromazine ne peut pas être recommandée comme traitement assoçié chez dex malades non psychotiques ayant une tuberculose pulmonaire, malgré son activité antimicrobienne *in vitro*. Cependant,d'autres produits similaires peuvent être découverts, ayant une plus grande activité antimicrobienne et moins d'effets toxiques.

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Case Reports

TEMPORARY PARALYSIS OF THE DIAPHRAGM[1]

Its Occurrence in Acute Pneumonia with Pleural Effusion

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Introduction

Temporary paralysis of the diaphragm is a rare occurrence in the course of acute pulmonary infections. In 1929, Humphrey and Sherwood (1) reported a case of streptococcic right lower lobe pneumonia in which complete paralysis of the right diaphragm was observed on fluoroscopic examination during the period of resolution. A chest roentgenogram four weeks later revealed complete clearing of the pneumonia and return to normal position of the right diaphragm. In a review of the literature at this time, Humphrey and Sherwood stated that no other documented cases of this type were to be found. In 1950, Freedman (2) reported six cases of complete diaphragmatic paralysis following pneumonia. Only two cases were discovered during the acute phase of the pneumonia; the others were detected shortly following resolution. In all but two, gradual return to normal function occurred. Freedman noted the apparent rarity of other documented cases of this sort in the literature.

A review of the literature at this time has also failed to produce other well-established instances of temporary complete paralysis of the diaphragm occurring in the course of acute pulmonary infection. For this reason, it seems appropriate to report a case in which this was observed.

Case Report

W. S., a 20-year-old white male army recruit, was in good health until mid-January, 1959, when he noted the gradual onset of malaise, easy fatigability, and cough. He was able to continue the basic training program despite these symptoms, but sought medical attention one week later when he developed fever and the cough became productive of moderate amounts of thick white sputum. Two days prior to admission to the hospital, he experienced right anterior chest pain of a pleuritic nature. There was no history of dyspnea or hemoptysis. On the day of initial hospitalization, January 29, 1959, hoarseness of the voice was first noted.

Physical examination on admission revealed a moderately ill, alert young male who complained of fever and cough. His temperature was 101°F., rectally, pulse 110 per minute and regular, and the blood pressure was 112 systolic, 70 diastolic in mm. of mercury. The posterior pharynx was moderately injected, and there was obvious hoarseness of his voice. Examination of the chest showed expansions to be symmetrical and full. Dullness to percussion and diminished to absent breath sounds were noted over the lower half of the posterior and lateral right chest. No rales or pleural friction rubs were heard. The heart was regular at 110 per minute, and no murmurs were audible. The remainder of the physical examination was within normal limits.

Laboratory findings on admission showed a leukocyte count of 28,800 per cu. mm. with a differential count showing 90 per cent neutrophils. The hematocrit was 42 per cent and the urinalysis was within normal limits. A sputum culture revealed alpha-streptococcus. In the initial chest roentgenogram (figure 1) of January 29, 1959, the right diaphragm measured 4.5 cm. higher than the left. There was an area of infiltration in the right midlung field, and some blunting of the right costophrenic angle was noted, suggesting some fluid accumulation.

Initial therapy consisted of bed rest, fluids, and 600,000 units of procaine penicillin intramuscularly every twelve hours. The temperature fell to normal on the third day, and symptomatic improvement occurred. A few coarse rales were heard over the midanterior right chest. A repeat chest film showed the presence of a rather large pleural effusion on the right side. A thoracentesis was done, but only a small amount of somewhat bloody fluid was obtained. A chest film on the fifth hospital day demonstrated some further increase in the amount of fluid in the right hemithorax. The patient was transferred to Valley Forge Army Hospital.

On admission on February 4, 1959, the patient was afebrile. Moderate hoarseness of the voice was again noted. Examination of the chest revealed findings compatible with a right pleural effusion. A thoracentesis was done in the right sixth interspace in the posterior axillary line, and 380 ml. of yellow, slightly turbid fluid were obtained. A number of small blood clots were also aspirated, and this seemed to confirm the impression that the original bloody fluid was of traumatic

[1] From the Pulmonary Disease Service, Valley Forge Army Hospital, Phoenixville, Pennsylvania.

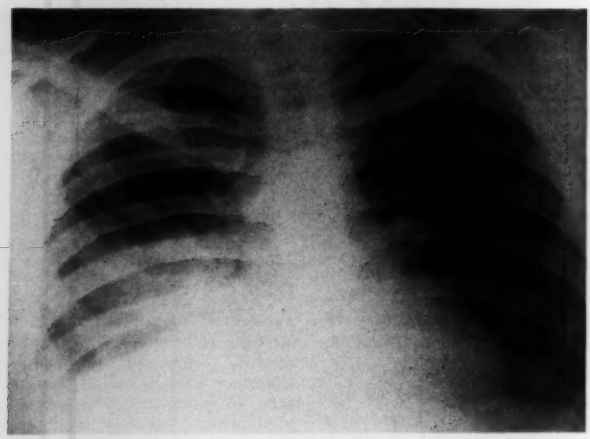


FIG. 1. Roentgenogram of January 29, 1959, at the time of initial hospitalization, showing the area of pneumonia in the right midlung field, elevation of the right diaphragm, and obliteration of the right costophrenic angle.

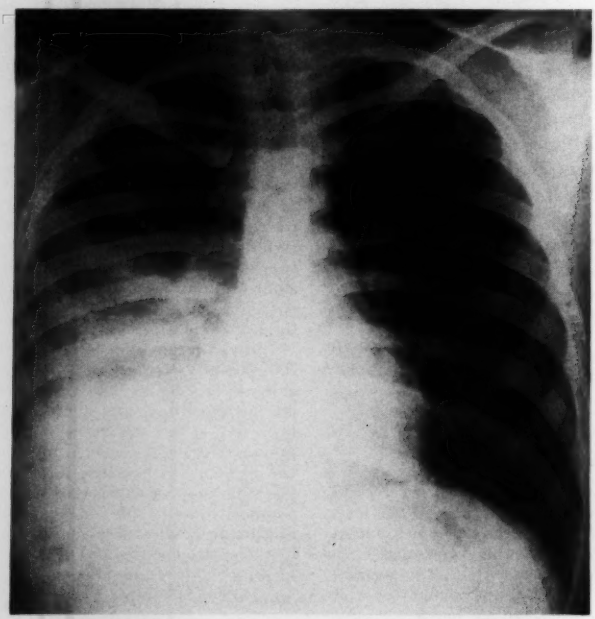
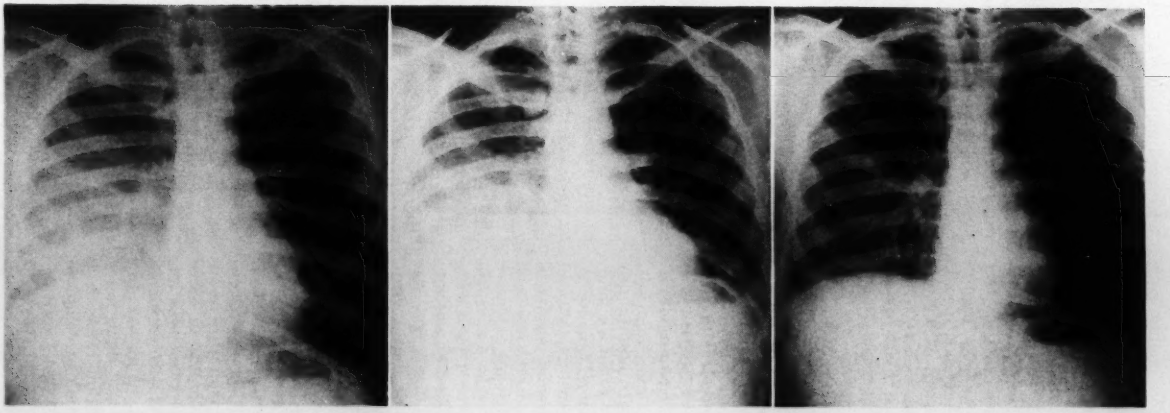


FIG. 2. Roentgenogram of February 13, 1959, again reveals elevation of the right diaphragm. Fluoroscopy at this time showed complete paralysis.

origin. Antimicrobial therapy was continued in doses of 300,000 units of procaine penicillin intramuscularly every eight hours. Indirect laryngoscopy on February 9 showed both vocal cords to move well, and moderate inflammation was seen. The patient continued to remain essentially afebrile except for occasional temperature elevations in the afternoon to 100.2°F. The hoarseness gradually subsided as his cough disappeared.

Initial laboratory studies at Valley Forge



FIGS. 3*A* and *B* (Left and Center). Inspiratory and expiratory roentgenograms of February 20, 1959, show a definite change in level of the right diaphragm, indicating return of function.

FIG. 4 (Right). Roentgenogram of March 30, 1959, shows resolution of the pneumonia and pleural effusion. The right diaphragm is somewhat elevated, but function was normal.

Army Hospital were as follows: Hematocrit and urinalysis were within normal limits. The leukocyte count was 14,750 per cu. mm. with 74 per cent neutrophils. All subsequent blood counts were within normal limits. Serial cold agglutinin titers showed a rise to only 1:16 on the thirtieth hospital day. Several blood cultures showed no growth. No ova or parasites were seen on stool examination. A battery of liver function tests, including a bromsulfalein test, alkaline phosphatase, prothrombin time, and albumin globulin ratio, were all normal. The cardiolipin microflocculation test showed no reaction. Examination of the pleural fluid obtained on February 5 showed a specific gravity of 1.020 and a total protein of 6.2 gm. per 100 ml. The leukocyte count was 2,000 per cu. mm. and erythrocytes totaled 1,000 per cu. mm. The fluid showed no growth on routine culture, and culture for *M. tuberculosis* was also negative. The intermediate-strength (0.001 mg. per ml.) and second-strength (0.05 mg. per ml.) PPD skin tests were negative, as were the histoplasmin, blastomycin, and coccidioidin intradermal tests (all 1:100 dilution). A sputum culture revealed alpha-streptococcus, Hemophilus, and a few colonies of *Staphylococcus aureus*, coagulase positive. A number of sputum and gastric washing cultures were negative for *M. tuberculosis*.

A repeat thoracentesis on the fifth hospital day was productive of only 50 ml. of yellowish fluid. A chest roentgenogram on February 13 (figure 2), several days following the procedure, showed considerable lessening in the degree of opacity in the right hemithorax. The area of infiltration in the right midlung field was slightly less extensive than on the original films. The right diaphragm was again seen to be markedly elevated, measuring 5.5 cm. higher than the left leaf. Chest fluoroscopy was performed, and the right diaphragm was found to be elevated and immobile during deep, full expiration. There was definite paradoxical movement on deep inspiration and inspiratory "sniffing." The left diaphragm was normal in position and motion.

To exclude the presence of an infrapulmonary effusion on the right, a right lateral decubitus film was taken after the patient had maintained this position for three hours. No change in contour was seen. Finally, a needle aspiration under fluoroscopic localization was attempted in order to detect free fluid in this area. None was obtained. It was therefore concluded with certainty that the right diaphragm was paralyzed and responsible for the density in the right lower lung field. Fluoroscopic examination three days later demonstrated the same finding of complete paralysis of the right hemidiaphragm.

The patient continued to show good recovery. Penicillin therapy was discontinued after twenty days and there was no recurrence of fever. Physical examination of the chest continued to reveal changes indicating elevation of the diaphragm. On February 20, twenty-three days after initial hospitalization and seven days after fluoroscopic demonstration of the right diaphragmatic paralysis, repeat fluoroscopy was done. The right diaphragm was still elevated, but now definite though decreased motion was detectable. This was most clearly seen on deep full expiration. Of interest to the examiners, however, was the observation that paradoxical motion still existed on inspiratory "sniffing." Inspiratory and expiratory chest roentgenograms (figure 3) taken the same day documented the change in position and contour of the diaphragm on deep inspiration. Bronchoscopy and a bronchogram of the right middle and lower lobes, obtained several days later, were within normal limits.

Final fluoroscopy was performed two weeks later, or twenty-four days after initial examination. At this time, the right diaphragm was of normal contour and only slightly elevated. Movement was brisk, and no paradoxical motion was seen. A final chest film (figure 4) was taken on March 30, 1959. The right diaphragm measured 3.5 cm. higher than the left. There was slight haziness over the right mid and lower lung fields, probably representing the residual of the pleural effusion. Pulmonary function tests (maximal breathing capacity, total vital capacity, and timed vital capacities) were all within normal limits.

The patient was returned to full duty status on May 14, 1959.

Discussion

Although the exact nature of the pulmonary infection in the patient is not clear, there is little doubt that the process was one of acute right-sided pneumonia and pleural effusion. Early antimicrobial therapy could have interfered with a precise bacteriologic diagnosis, for penicillin therapy preceded culturing of the sputum by about eight hours. It is also possible that this was a viral pneumonia or, less likely, a pulmonary infarction. The important feature, however, was the documentation of the transient, complete paralysis of the diaphragm on the affected side. The initial chest roentgenogram showed evidence of some elevation of the right diaphragm. The rapidly developing pleural effusion obscured this for a time but, following thoracentesis and resolution of the infection, it was again seen that a markedly elevated right diaphragm was present. It was thought that this degree of elevation was somewhat unusual in pneumonia, and further investigation by fluoroscopic techniques demonstrated conclusively that complete paralysis was present. This finding alerted the examiners to the possibility of such diagnoses as intrathoracic neoplasm, tuberculosis, or even those neurologic disorders which may produce a phrenic nerve paralysis.

A review of a roentgenogram taken in December, 1958, prior to induction into the Army, showed the right diaphragm to be of normal contour and position, thus excluding the possibility of eventration. Repeated observation confirmed the presence of paralysis. Within seven days after the initial discovery of paralysis, there was faint though definite motion detected on the affected side. This was followed by gradual return of complete function and, within twenty-one days, movement was normal. The final chest film showed a slightly elevated right diaphragm of normal contour.

In discussing the features of paralysis of the diaphragm, a clear distinction should be made between this and the other types of abnormal function of the diaphragm. These would include weakness or paresis, eventration, and fixation of the diaphragm. True paralysis is characterized by elevation, immobility, and paradoxical motion. The degree of elevation need not be great immediately following the onset of paralysis, although progressive atrophy of the musculature will eventually lead to notable elevation. Furthermore, adhesions from previous inflammatory disease may restrict passive rise in level. Immobility may be detected on fluoroscopic examination as the patient performs slow full inspirations and expirations. Chest roentgenograms taken in these positions are also useful in demonstrating immobility. The mechanism of paradoxical motion will be discussed subsequently.

Paralysis of the diaphragm implies impairment of function of a phrenic nerve, which is considered to supply the sole motor fibers to the corresponding leaf. An exception to this statement lies in congenital eventration of the diaphragm, when the defect probably exists in the leaf itself rather than in the motor nerve supply. There are a number of recognized etiologies of phrenic nerve dysfunction. Lesions of the cervical cerebrospinal cord, such as poliomyelitis, tumors, and trauma, may destroy the motor areas of the third and fourth and often fifth cervical nerves, from which the phrenic nerve has its origin. Brachial plexus injuries (3) may also result in its paralysis. As the phrenic nerve passes through the thoracic cavity in proximity to the mediastinum, it is vulnerable to destruction by neoplasms involving the hilar structures or by tuberculous lymph nodes in the same location. The prognostic implications of paralysis of a diaphragm in bronchogenic carcinoma are well known. Case reports have also noted phrenic nerve paralysis to follow herpes zoster involving cervical cord roots (4) and to result from diphtheritic neuritis of the phrenic nerve (5).

In a review of 30 cases of unilateral phrenic nerve palsy, Briscoe (6) mentioned aortic aneurysm and syphilis as other etiologies. Paralysis need not involve all portions of the diaphragm. Abeles and Leiner (7) reported this finding in a patient who had had poliomyelitis involving the cervical cerebrospinal cord. The right diaphragm showed paralysis confined to the anterior and medial portions. They postulated that the patchy involvement of the motor areas had spared those nuclei from which the motor fibers to the intact portions arose. Therapeutic phrenic nerve crush, although not used as often now as in years past, is another cause of diaphragmatic paralysis.

Weakness or paresis of the diaphragm is a more common finding than paralysis. This type of abnormality is characterized by elevation of the diaphragm with diminished, though definite, motion. Paradoxical motion, which is essential in the diagnosis of complete paralysis, is not seen. A probable exception to this was illustrated in the case reported. Here, it was seen that, during the phase of early return of function of the affected diaphragm, paradoxical motion persisted, despite definite though diminished inspiratory and expiratory motion. This will be considered later. A number of disease states are associated with weakness and some elevation of the diaphragm. Pneumonia of lobar extent, particularly with lower lobe involvement, and lower lobe atelectasis are perhaps the most familiar. In pneumonia, a secondary "diaphragmitis" has been postulated to result in transient weakness and elevation. This may also be due to partial atelectasis of the involved lobe and the elevation and paresis representing both a passive rise in level and loss of tone. Comroe and associates (8) and Stanton and Miller (9) reported cases of transient weakness of the diaphragm following tetanus antitoxin administration. A "phrenic neuritis" was thought to be responsible. Meyler and Huizinga (10) observed six cases of transient weakness of the right diaphragm shortly following cholecystectomy. In discussing the syndrome of acute primary diaphragmitis, Joannides (11) noted that the affected leaf showed elevation and

either diminished or absent motion. It was not stated if true paralysis was seen.

There has been some disagreement in the literature over the use of the term "eventration of the diaphragm." The term does not imply any specific type of functional impairment, for, as pointed out by Reed and Borden (12), motion may be present or absent, and paradoxical motion is not always seen. Laxdal and co-workers (13) state that paralysis is present in about 50 per cent of cases. A differentiation is made between the congenital type, which is most often on the left side and probably the result of some developmental defect in the muscle fibers, and the acquired type of eventration. The latter term applies to the paralyzed diaphragm resulting from phrenic nerve destruction. The term eventration, therefore, is not used to denote an abnormality of function, but an abnormality of position. The etiology may be either congenital or acquired.

The final type of dysfunction is that characterized by elevation aand fixation of the diaphragm. Here, immobility is found, but paradoxical motion is not seen A fixed diaphragm is one in which mechanical factors interfere with free movement. This may be seen following the formation of dense adhesions resulting from a neglected pleural effusion, an empyema, repeated therapeutic pneumothorax, or a thoracotomy. A well-known cause is that of a subphrenic abscess. A fixed, elevated diaphragm may most closely resemble true paralysis. As stated, the lack of paradoxical motion will help to differentiate the two.

If the term "paralysis" of the diaphragm is reserved for only those instances in which elevation, immobility, and paradoxical motion are seen, then distinction from the other types of dysfunction is not difficult. Careful fluoroscopy, with attention to the proper techniques in order to minimize any radiation hazard, is the most important procedure in this evaluation.

One of the more interesting features of the present case was the observation that paradoxical motion was seen at a time when normal, though diminished, movement was present. A similar finding was reported by Wynn-Williams (14), who observed five cases of what he termed paresis of the diaphragm; in two of the instances, definite paradoxical motion was seen on inspiratory "sniffing." Freedman (2) made a similar observation. This emphasizes the point that paradoxical motion alone does not denote paralysis but, if elevation and immobility are also seen, then the term correctly applies. The mechanism of paradoxical motion may be briefly summarized as follows: normally, deep and full inspiration produces an increase in intra-abdominal pressure as both diaphragms descend. If one leaf is functionless and completely lacking in tone, the increase in intra-abdominal pressure will cause its passive upward movement. This is the paradoxical motion. If inspiratory "sniffing" is done in place of slower inspiration, the increase in pressure is more acute and a more impressive upward motion is observed fluoroscopically. This maneuver is commonly employed to detect paradoxical motion. If the dysfunction is transient and as tone starts to return to the affected diaphragm, there will be some motion detected on full inspiration and expiration. But sudden changes in pressure still cannot be overcome, and paradoxical motion will persist on the "sniff test." As further return of tone occurs, however, a point will be reached at which movement is downward on inspiration and paradoxical motion is no longer demonstrated. This sequence would seem to explain the events noted in the case reported.

Couch (15) and Freedman (2) have reported a series of cases in which complete paralysis of the diaphragm was noted following recovery from pneumonia. Most of the patients were asymptomatic and, in all but a few, complete return of function was seen to occur in a period of several weeks to several months. These cases differ from the one here reported in that the onset was not documented during the acute phase of pneumonia (with two exceptions) and recovery was much slower. It does seem logical to assume, however, that the basic phenomenon was similar to the one in the present case. Both writers speculated that this occurrence is probably more common than is generally reported. A "phrenic neuritis" has been postulated as a possible etiology of the temporary paralysis of the phrenic nerve. An acute diaphragmitis with complete loss of function has also been mentioned. The former theory is lent some support by the fact that in one of the cases reported by Freedman (2) there was a concomitant temporary paralysis of the ipsilateral vocal cord.

The incidence of temporary paralysis of the

diaphragm during acute pulmonary infection is probably greater than has been reported. It may not be detected if elevation of the diaphragm on the affected side is not great. Furthermore, the transient nature of the process may account for its not being discovered. If fluoroscopic examination was made in all patients with elevation of the diaphragm in acute pulmonary infections, then perhaps other documented cases could be added to the one here reported.

Summary

A case is reported in which a complete paralysis of the right diaphragm was observed in the course of an acute right-sided pneumonia and pleural effusion. As the infection subsided, gradual return of function took place. Paradoxical motion of the involved leaf was seen to persist for a brief period after motion of the diaphragm returned. The etiology of the temporary paralysis is not known, but a "toxic neuritis" involving the phrenic nerve has been postulated. It seems likely that this occurrence is not as rare as the literature would indicate.

Sumario

Parálisis Temporal del Diafragma. Su Ocurrencia en la Neumonía Aguda con Derrame Pleural

Preséntase un caso en el que se observó parálisis total del lado derecho del diafragma durante la evolución de una neumonía aguda y derrame pleural del mismo lado. Al ceder la infección, tuvo lugar el retorno gradual de la función. Se observó que, después de reaparecer el movimiento del diafragma, persistió por un breve período de tiempo el movimiento paradójico de la hoja afectada. No se conoce la etiología de esta parálisis temporal, pero se ha presupuesto la existencia de una "neuritis tóxica" que afecta el nervio frénico. Parece probable que este fenómeno no sea tan raro como indica la literatura.

Resume

Paralysie temporaire du diaphragme. Sa fréquence dans les pneumonies aiguës avec pleurésie

L'auteur rapporte un cas dans lequel il observa une paralysie de l'hemi-diaphragme droit au cours d'une pneumonie aiguë du coté droit avec réaction liquidienne dans la plèvre. Au fur et à mesure que l'infection diminua, le diaphragme recouvra graduellement sa fonction. On observa que des mouvements paradoxaux de la partie atteinte persistèrent peu de temps après que le diaphragme ait récupéré sa motilité. L'étiologie de cette paralysie temporaire n'est pas connue, mais on a supposé qu'il s'agissait d'une "névrite toxique" intéressant le nerf phrénique. Trés vraisemblablement, sa fréquence n'est pas aussi rare que la littérature veut le dire.

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THE SUCCESSFUL TREATMENT OF PULMONARY COCCIDIOIDOMYCOSIS WITH INTRAVENOUS AMPHOTERICIN B[1]

B. J. LONGLEY AND J. T. MENDENHALL

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INTRODUCTION

Fungal diseases have been particularly resistant to control by chemotherapeutic agents used for bacterial infections. Newcomer and associates (1) have reviewed the chemotherapy of coccidioidomycosis in man and have pointed out that a number of agents appear to be of some benefit in some patients, but that none of the agents are outstanding. Such a situation leads to the belief that these agents are of low potency against the infection and that host resistance is an important factor in those persons who do recover.

Fiese (2) reported a case of coccidioidomycosis in which oral amphotericin seemed to be of benefit, and Littman (3) reported two cases in which there was a striking response to intravenous amphotericin B. The oral preparation had failed to produce either satisfactory blood concentrations or clinical response in one of these patients. The intravenous preparation gave a satisfactory response in both of them.

The present writers have had the opportunity of observing a patient with pulmonary coccidioidomycosis, empyema, and bronchopleural fistula following a resection. Hydroxystilbamidine, nystatin, and oral amphotericin B were of little benefit, but the response to intravenous amphotericin B was striking. This patient was cited by Dickie (5), but has not been reported in detail.

CASE REPORT

The patient was a 28-year-old white male living in Phoenix, Arizona, at the onset of his symptoms in March, 1955. At first he noticed easy fatigability associated with a gradual weight loss of 30 pounds, and then he developed a spontaneous pneumothorax associated with a pleural effusion on June 17, 1955. A closed thoracotomy was performed and fluid was aspirated. The tube was left in place for nine days, during which time the patient was febrile and the lung expanded poorly. Antimicrobials were given for a period of three weeks. Four weeks after the onset of symptoms, fever and a productive cough returned, with the production of 90 to 120 ml. of sputum per day. Two months after the onset of the pneumothorax and after preoperative treatment with antimicrobials, a left thoracotomy was done. The clinical diagnosis was an abscess, which required removal of the left lower lobe and part of the lingula. The pathologic diagnosis was fibrocystic tuberculosis (the organisms being present on periodic acid-Schiff tissue stains) and coccidioidomycosis, the latter being grown on culture.

The postoperative course was characterized by toxicity, and a bronchopleural fistula developed. One of the thoracotomy tubes was left in place for drainage, and, on September 12, 1955, the patient was admitted to the Veterans Administration Hospital, Madison, Wisconsin. At this time, he was acutely ill. The skin tests for histoplasmosis, coccidioidomycosis, and tuberculosis were all negative. The tuberculosis skin test included OT and second-strength PPD. The complement-fixation test for coccidioidomycosis was positive in a dilution of 1:32. Cultures from the drainage yielded *Coccidioides immitis*, and stilbamidine therapy was started. A chest roentgenogram (figure 1) revealed the empyema cavity on the left and scattered disease on the right. The latter disease on the right side showed some progression during stilbamidine therapy.

On October 11, 1955, sections of ribs seven, eight, and nine were resected and the skin was sewn to the pleura, establishing dependent and adequate drainage. Stilbamidine was continued to a total dose of 8 gm., and then stopped. With the discontinuance of stilbamidine his appetite improved. Stilbamidine was restarted and a total dose of 1.8 gm. given and then stopped, because of a direct Van den Bergh reaction of 0.9 mg. The empyema cavity during all of this time remained grossly purulent, and the second-strength PPD reaction remained negative. On August 18, 1956, thirteen months after his spontaneous pneumothorax, the patient was considered for surgery; but any procedure was deferred at that time, since the disease process was considered to be too active. The coccidioidin skin test remained negative. The patient showed reactivation of the parenchymal disease on the right side on October 23, 1956 (figure 2), and this cleared adequately by January 23, 1957 (figure 3).

The patient was discharged from the hospital on October 24, 1956, with a diagnosis of coccidiodomycosis and a bronchopleural fistula. In view of

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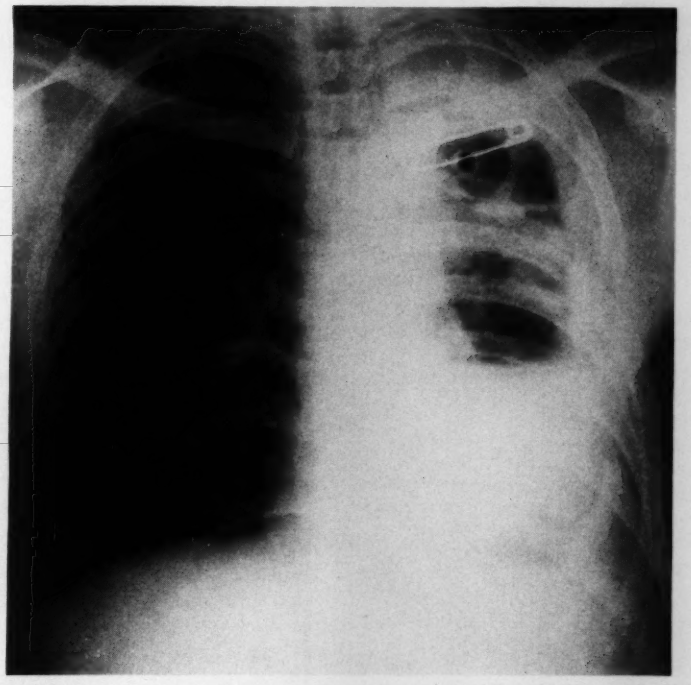

FIG. 1. Admission roentgenogram, September 12, 1955, showing bilateral parenchymal involvement, empyema, and a drainage tube on the left.

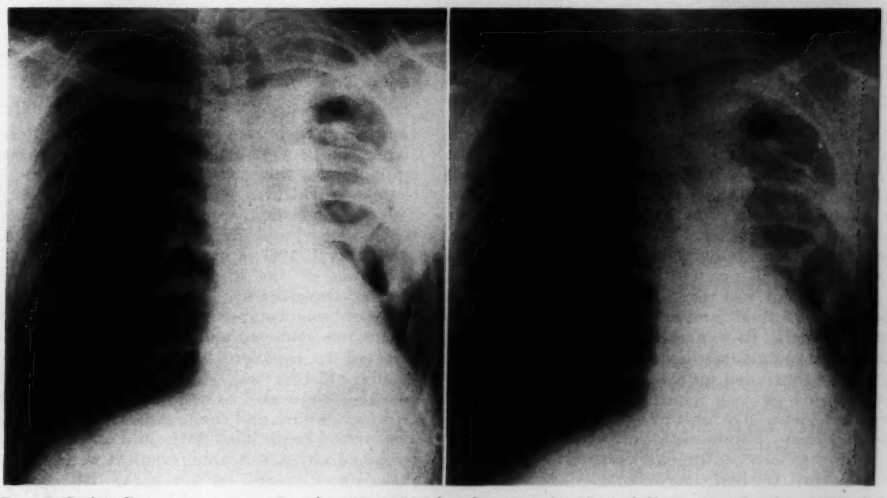

FIG. 2 (Left). Roentgenogram, October 23, 1956, showing reactivation of disease at the tip of the right third rib.

FIG. 3 (Right). Roentgenogram, January 23, 1957, showing clearing of lesion at the tip of the right third rib.

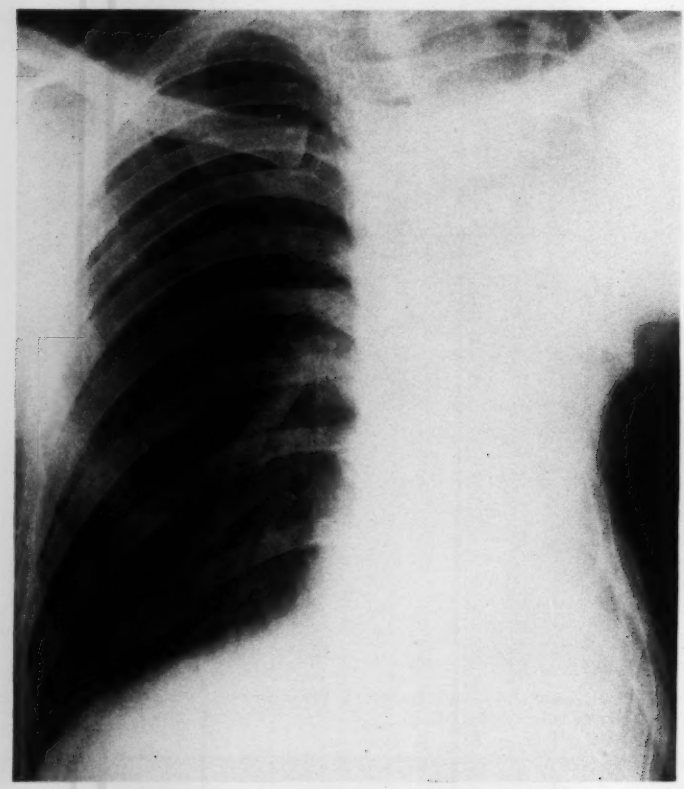

FIG. 4. Roentgenogram, July 17, 1958, eleven months after discharge, showing post-pneumonectomy thoracoplasty on the left and stable lesions on the right side.

the negative tuberculin reaction and the atypical appearance of the cells on the tissue stains, the diagnosis of tuberculosis could not be confirmed. While at home, the patient took nystatin, two million units daily for about four months. This produced no significant change in clinical course, and the cultures remained positive for *Coccidioides immitis*.

The patient was readmitted to the hospital on February 15, 1957, and on February 21, 1957, oral amphotericin B was started. The dosage was increased to 2 gm. per day without signs of toxicity and was continued for about two months. During the period of two months' treatment by oral amphotericin, five cultures negative for *Coccidioides immitis* were obtained, whereas they had previously been continuously positive.

On April 16, 1957, intravenous amphotericin B was started and continued daily for a few days and then was given three times a week. The dose was increased to about 35 mg. for a patient weighing 54 kg. Toxic symptoms were nausea and vomiting, which necessitated limiting the dose to 35 mg. Three days after starting the intravenous amphotericin there was one positive culture, and thereafter cultures were continuously negative. The empyema fluid, previously grossly purulent, became clear, and drainage decreased in amount.

On July 1, 1957, approximately two years after the onset of the disease and ten weeks after starting intravenous amphotericin, a pneumonectomy was completed on the left side. The pleura was removed and the remaining ribs were resected to complete a thoracoplasty, and the drainage fistula was closed. Cultures from the specimen revealed *Coccidioides immitis*, but growth was slow and the organisms were atypical. The wound space filled with fluid and the patient remained afebrile. Amphotericin B was continued three times a week for six weeks. All skin tests were negative, as was the complement-fixation test for coccidioidomycosis. The patient was discharged from the hospital on August 30, 1957, and was followed as an outpatient. His chest

roentgenogram remained stable (figure 4) and the complement-fixation tests remained negative. Approximately three years after the onset of the coccidioidomycosis, the patient returned to Phoenix to resume his job as a car salesman.

Discussion

This patient provides clear-cut evidence of the control, but not the "cure," of coccidioidomycosis by intravenous amphotericin. The fact that cultures were positive for *C. immitis* on only one occasion after starting treatment with the intravenous preparation, together with the "clearing up" of the empyema cavity, convinced all who observed the patient of the benefit of intravenous amphotericin in this instance. The failure of stilbamidine and of nystatin was equally obvious.

Certain features of the clinical course of the disease were of interest. The onset as a hydropneumothorax, while not uncommon, certainly is not the usual situation when a patient is first seen. The reactivation of the disease in the right lung and its control by body resistance alone was a feature not fully appreciated at the time it occurred. There are certain areas in which our knowledge is inadequate; particularly is this true with regard to optimal duration of therapy both preoperatively and postoperatively. The preoperative therapy was continued beyond the point of cultures negative for *Coccidioides immitis* and stable lesions on the roentgenogram. This period might conceivably have been shortened, but was continued for a full two and a half months because of the actual difficulty of determining if the lesion itself was pathologically stable. In the absence of evidence of the emergence of drug-resistant organisms, it is believed that no harm was done and that actual benefit was derived from continuing the full two and a half months of treatment. It is possible that continued treatment beyond this time may have "sterilized" the lesions.

TABLE 1

Susceptibility of C. Immitis to Amphotericin B

	Drug Concentration (γ per ml.)			
	0.0	0.5	1.0	5.0
Growth at 4 days	++++	0	0	0
Growth at 10 days	++++	++	++	No growth at 21 days

Cultures isolated from the surgical specimen showed the same degree of drug resistance as did the original culture.

The susceptibility of *C. immitus* to amphotericin is indicated in table 1. Since the cultures became negative early in the course of treatment, it was not possible to measure *in vitro* resistance to the drug. However, studies on the culture isolated from the surgical specimen indicated that significant *in vitro* resistance had not developed after two and a half months of treatment.

The amount of amphotericin in the blood was not determined, but was assumed to approach that reported by Littman (3) and Louria (4), although a somewhat lower dosage level was used in the present case. However, nausea and vomiting could not be controlled when higher dosage levels were used. Other toxic reactions, such as fever or renal damage, were of no significance.

The decision as to the duration of postoperative chemotherapy was purely arbitrary and chemotherapy was continued for two months. It would have been continued beyond this point if the complement-fixation test had been positive.

Summary

A case is presented in which pulmonary coccidioidomycosis, empyema, and bronchopleural fistula responded satisfactorily to intravenous amphotericin B. After ten weeks of intravenous amphotericin B therapy, a left pneumonectomy was completed. The organisms recovered from the resected lung showed the same susceptibility to amphotericin as was found in the original pretreatment cultures. Oral amphotericin, hydroxystilbamidine, and nystatin were without clinical effect.

Sumario

El Tratamiento Acertado de la Coccidioidomicosis Pulmonar con Anfotericina B por Vía Venosa

Preséntase un caso en el que la coccidioidomicosis pulmonar, el empiema y una fístula broncopleural respondieron satisfactoriamente a la anfotericina B por vía venosa. Al cabo de diez semanas de terapéutica intravenosa con anfotericina B, se completó una neumonectomía izquierda. Los microbios obtenidos del pulmón resecado revelaron la misma susceptibilidad a la anfotericina que se había observado en los cultivos primitivos de antes del tratamiento. La anfotericina, la hidroxistilbamidina y la nistatina no mostraron efecto clínico por vía oral.

Resume

Succès du traitement de la coccidioidomycose pulmonaire par l'amphotéricine B intraveineuse

Les auteurs présentent un cas de coccidioidomycose pulmonaire avec empyème et fistule bronchopleurale qui a répondu d'une façon satisfaisante à l'amphotéricine B intraveineuse. Aprés dix semaines de traitement par l'amphotéricine B intraveineuse, une pneumonectomie gauche fut pratiquée. Les germes prélevés dans le poumon réséqué montrèrent la même sensibilité à l'amphotéricine que celle qui fut trouvée dans les cultures originales avant le traitement. L'amphotéricine orale, l'hydroxystilbamidine, et la nystatine n'eurent aucun effet cliniquement.

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Editorials

Tuberculosis Chemotherapy as a Public Health Measure

The proper exploitation of antimicrobial therapy as a *public health measure* for the control of tuberculosis in this country is the major recommendation of the Arden House Conference, presented elsewhere in this issue of the REVIEW. It is of interest that, quite independently, Dr. Israel of Philadelphia had submitted an editorial for the REVIEW (*see* below) in which he presents substantially the same conclusions as the conferees. Moreover, also quite independently, Dr. Chapman of Dallas, Texas, has presented recommendations as to how certain aspects of the tuberculosis control movement should be modified to fit the epidemiologic situation as it exists today (*see* Correspondence below). In another Letter to the Editor, cited by Dr. Israel, Dr. Oatway of Altadena, Calif, urged the adoption of the practice of administering isoniazid for one year to patients with arrested tuberculous lesions of the lung who had never received chemotherapy. With due respect to the proper ratio of swallows to summer, therefore, there seems to be abroad in the land a certain amount of healthy scrutiny and critical reappraisal of the U. S. antituberculosis campaign as it exists today. And, it is certainly no discredit to the leadership of both the Public Health Service and the National Tuberculosis Association, who decided jointly to convene the Arden House Conference, to say that in so doing they were perceptively reflecting the spirit of the times.

The major recommendation of the Conference, that chemotherapy be employed as a *public health measure*, quite properly calls to mind certain important questions. Naturally, these questions (and many others) were carefully considered by the conferees. As the present writer was an active participant in these proceedings, there can be no pretense of editorial disinterestedness in this review of what seem to be the major questions involved.

First: *Can a nation-wide public health attack on an infectious disease be based on the chemotherapy of individual instances of the disease?*

This is precisely what *was* done in the case of syphilis in the nation-wide campaign led by Dr. Thomas Parran of the Public Health Service with the able assistance of two nongovernmental physicians, Dr. John Stokes and the late Dr. Joseph Earle Moore. Thus, the Arden House concept is recognizable as an accepted public health method comparable with the other methods for the wide-scale public health control of infectious diseases. Practicability is the essence of a public health campaign. If a disease is spread by water, one disinfects the water. If introduction of the disease to a country can be prevented by effective immunization of its people, one vaccinates the people. When the microbes of the disease are not very accessible to attack outside the body, one attacks them inside the body. And, to sterilize most or all of the tubercle bacilli in the body of a patient is a perfectly attainable goal in a great majority of instances.

Second: *Who would have to be treated; and should patients be treated whether they wish to receive treatment or not?*

Such a program presumably would be organized in successive stages. The first to be treated would be all persons with active tuberculous infection, i.e., all those with disease in the transmissible or potentially transmissible form. The next priority would be assigned to patients with demonstrable pulmonary lesions that were never treated with drugs and, hence, whose future stability remains in question. Next in priority would probably be *selected groups* of tuberculin reactors who present no other evidence of tuberculous disease. Included in such groups would be infants and preschool children, perhaps certain adolescents, and other such groups that could be shown to be a special risk.

Certainly, no one whose tuberculosis is not in a transmissible form should receive treatment against his wishes, although most persons with tuberculosis—as those with syphilis—actively desire treatment. In the case of persons with highly contagious forms of tuberculous infection, it is generally accepted today that they should be required to take treatment until their disease is in a nontransmissible stage.

Third: *Are we not already doing essentially what is in the recommendation without observing a striking, rapid fall in tuberculosis morbidity?*

In actuality, we are *not* doing what is in the recommendation, as judged by the information available. For example, the U. S. Public Health Service estimates on the basis of field studies that, in 1956, about 800,000 Americans with tuberculosis needed medical supervision in the public health interest; yet only about one-half of this number were actually receiving such supervision.

Fourth: *Who would supervise the treatment?*

The treatment of tuberculous patients in the hospitals and sanatoriums would be as it is now,

the responsibility of the chest physicians in charge of such clinical services. In addition, as discussed elsewhere (McDERMOTT, W.: J. Chronic Dis., 1955, *2*, 234), we are witnessing on an ever-widening scale the transfer of the responsibility for the long-term management of the tuberculous patient to the internist and general practitioner. Such a transfer of responsibility for a major disease from one group of physicians to another is unprecedented and represents an unsought consequence of our technologic gains. The problems that this transfer is creating are quite formidable, but they must be faced in any case. Indeed, it is not too unlikely that their solution may prove to be easier and considerably more orderly if a nationwide antituberculosis campaign based on the Arden House concept becomes a reality.

Fifth: *Does the Arden House recommendation imply the abandonment of the hospital or sanatorium treatment of pulmonary tuberculosis?*

Certainly not. There are large numbers of patients who for one reason or another present situations which can only—or can best—be treated in the hospital or sanatorium. All tuberculosis specialists are agreed, however, that the portion of the treatment represented by chemotherapy should be continued for a much longer period than even the most conservatively managed period of hospitalization. Moreover, many of the leading authorities cited by Dr. Israel, below, agree that an impressive proportion of the patients with tuberculosis can be treated entirely at home. The scientific basis for such home treatment has been provided by the carefully conducted study by Hirsch and his associates at the Rockefeller Institute (Am. Rev. Tuberc., 1957, *75*, 359) and the more recent study by the Tuberculosis Chemotherapy Center in Madras, India (Bull. World Health Organ., 1959, *21*, 51). In both studies it was shown that, in the presence of theoretically adequate chemotherapy, it was not possible to detect any influence of physical activity on the generally favorable course of the healing process.

Sixth: *Does a chemotherapy for tuberculosis exist that is both sufficiently effective and safe to warrant employment in the treatment of all cases of tuberculosis as a public health measure?*

In the availability of the three regimens, isoniazid–streptomycin, isoniazid–PAS, and isoniazid alone, we have three highly effective chemotherapies available for use in different sets of circumstances. There has been no serious debate as to the therapeutic effectiveness of daily streptomycin and isoniazid or isoniazid–PAS, although there are certain limitations to the broad-scale usage of the former regimen. Likewise, there appears to be no serious debate as to the wisdom of employing isoniazid alone in certain limited circumstances, for example, in disease prophylaxis, i.e., the treatment of the tuberculin reactor without obvious disease. Whether the employment of isoniazid alone can be safely broadened from this base *is* a matter of serious debate. With respect to safety, it can be stated with confidence that isoniazid is one of the least toxic drugs in general use in all therapeutics. The toxicities of PAS and of streptomycin are appreciably greater, but are now so well defined that, with simple management, virtually all serious toxicity can be easily avoided. The point for emphasis, therefore, is that, even if the use of isoniazid alone is restricted to chemoprophylaxis, we have available today enough satisfactory tuberculosis chemotherapies to make their employment as a public health measure a feasible procedure.

Seventh: *Is there any urgency in starting a campaign for the employment of chemotherapy of tuberculosis as a public health measure?*

A very definite urgency exists because there can be no real assurance that, whatever the chemotherapy, there will not emerge a minority, however small, of drug-resistant tubercle bacilli with the full capability for producing disease. Indeed, it is not always appreciated that this occurs in patients treated with multiple-drug therapy, such as isoniazid–PAS, as may be seen from study of two recently reported series (PHILLIPS, S.: Am. Rev. Resp. Dis., 1959, *80*, 641; GRAHAM POOLE and STRADLING: Brit. M. J., 1960, *1*, 161). Whereas there is no evidence as yet that such drug-resistant tubercle bacilli are causing appreciable numbers of new infections, yet all biologic experience indicates that we are "working against time" unless equally powerful chemotherapies are developed. The period of time involved may seem rather long—perhaps ten years or more—but it has very definite limits. Thus it is that, if chemotherapy is to fulfill its full potential to bring tuberculosis under control, the public health attack must be made *now* before the current irregular and disorganized treatments have swelled this minority of drug-resistant bacilli to the dangerous point. In this respect, the situation is not unlike that in the current global campaign against malaria which admittedly has to be based on the principle of "working against time."

Eighth: *Will the patients take the pills with sufficient faithfulness and for a sufficiently long period to ensure sterilization of most or all of their tubercle bacilli?*

For a person who feels completely well to take a pill daily for a long period of time is a strangely difficult accomplishment in any society. Yet, to a much greater extent than was ever the case before, the advantages of modern medicine are dependent

on the assumption of this responsibility by the patient. Obviously, this is a question with broad public health implications quite aside from the questions of tuberculosis control, and should receive very extensive study. The available information is limited, but thus far it indicates that perhaps people are more reliable in this respect than the physicians anticipate. For example, in the USPHS studies of chemoprophylaxis in children who were tuberculin reactors, the isoniazid was not actually *administered* daily by the investigators, but its ingestion was *prescribed*. Of more than 1,000 children for whom isoniazid was *prescribed*, there were two instances of the development of tuberculous disease in contrast to the twenty-eight instances of such disease in a comparable group for whom the placebo was *prescribed*. Deuschle's experience with the Navajo (Am. Rev. Resp. Dis., 1959, *80*, 200) likewise would indicate that, in a properly organized program, enough people are reliable to make the program a successful one.

* * *

The ninth question is in many ways the biggest one of all, namely: *Is it reasonable to suppose that all the many forces, facilities, and people in the health movement in the United States could be successfully mobilized to organize a nation-wide campaign in which the chemotherapy of tuberculosis were made a public health measure?*

It would be unrealistic to minimize the enormous effort that would be involved in getting the campaign started. Such a movement would have to gain the enthusiastic cooperation of: the medical profession, both as individuals and as members of organizations; the municipal, county, state, and national health officers; the lay and professional members of the voluntary health movement; and the governmental bodies that have responsibility for the appropriation of money. What would be needed for leadership would be a government and nongovernment team like the old Parran-Moore-Stokes team that so successfully mobilized such a program against syphilis.

If the present writer understands the thinking of the Arden House conferees correctly, they are saying "If you are really serious about the desire to eliminate tuberculosis as a force in the United States, then this is the most practicable and scientifically sound way it can be done." As with so many other questions, whether society chooses to put itself to trouble for the attainment of a goal is another matter. The responsible leaders in the U. S. Public Health Service and the National Tuberculosis Association, with its American Trudeau Society, are indicating, in backing this recommendation, that they are serious. It remains for us to show that we are serious too.

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The Next Step in the Campaign against Tuberculosis

Tuberculosis in 1959 bears little resemblance to the disease we knew ten to twenty years ago. The epidemiology of the disease, case-finding techniques, methods and results of treatment—everything but the fact that infection with *Mycobacterium tuberculosis* is a prerequisite for the disease—seem different.

The death rate from tuberculosis in the United States has fallen from 49.1 per 100,000 persons in 1938 to 30.0 in 1948 to an estimated 7.0 in 1958. It is frequently argued that death rates are not an accurate index of the tuberculosis problem. This may be true, for most of the deaths attributed to tuberculosis are actually due to the sequelae of old tuberculous lesions or to incidental diseases which occur in tuberculous patients.

The prevalence of tuberculous infection, however, can be determined precisely by the tuberculin test; and the decline in tuberculous infection has been almost as precipitous as in the death rate. Studies of U. S. Navy recruits have shown a 45 per cent drop in the percentage of tuberculin reactors from 1950 to 1957, from 9 per cent to 5 per cent.

Much has been made in the past few years of the failure of tuberculosis morbidity to decline proportionately. The number of newly reported cases in many cities and states for a time increased and has only lately begun to fall. In Philadelphia, for example, the number of cases reported was 1,879 in 1938, 2,894 in 1948 and, despite increased case-finding facilities, was 2,420 in 1958.

One recent study casts considerable light on our changing diagnostic practices and standards. Disturbed by the reports of increasing incidence of tuberculosis in children in California, Ohio, and New York, Schneider and Drummond[1] undertook a study of all cases reported to the Los Angeles Department of Health in persons less than fifteen years of age. The number reported rose from 84 in 1951 to 201 in 1955. Careful review indicated that there were 14 fatal cases in 1951 while there were

[1] SCHNEIDER, R. M., AND DRUMMOND, E. E.: Am. Rev. Tuberc., 1957, *76*, 579.

only 3 fatal cases in 1955. Miliary, meningeal, extrapulmonary, and reinfection cases totaled 19 in 1951 and 18 in 1955. The increase in total "morbidity" during these years was accounted for entirely by a rise in reports of primary tuberculosis. Analysis of these cases, numbering 44 in 1951 and 133 in 1955, revealed that, in the earlier year, 42 of the patients had had their disease discovered as a consequence of symptoms. In 1955, on the other hand, a majority were asymptomatic and were found only because of intensive community surveys. Thus, the rising incidence of tuberculosis proved to be but an apparent increase due to an increase in reporting.

The change in the epidemiology of tuberculosis has been not merely a quantitative one, but a qualitative one. One disputed question in the past was whether tuberculosis resulted from exogenous or endogenous infection. In fact, it has been due to both, but there can be little doubt that adult tuberculosis twenty years ago was principally the result of adult exposure to tuberculosis. The high incidence of tuberculosis among nurses and physicians bore witness to this and, because tuberculin-negative subjects were more likely to develop tuberculosis, BCG vaccination seemed a desirable protection for the minority of young adults who were still tuberculin negative. However, more recent studies[2] have shown that at present tuberculosis develops principally among subjects who are tuberculin positive upon coming under observation. Most tuberculosis now results not from exogenous infection, but from breakdown of pre-existing disease. Increasingly, therefore, we must focus our attention on (*1*) those who had extensive tuberculosis treated successfully in the pre-isoniazid era, and (*2*) those with small roentgenographic lesions which appear to be of questionable clinical significance or which appear to be entirely healed. Both of these will be found predominantly in older persons who lived at a time when tuberculous infection was widespread.

For a quarter of a century emphasis in case finding was placed upon increasing use of mass roentgenography as the best means of detecting pulmonary tuberculosis. The development of relatively inexpensive methods of photofluorography made possible city-wide surveys in which enormous populations were examined. Further utilization of this approach has been slowed by two current developments: first, apprehension concerning the untoward effects of irradiation; and, second, recognition of the increasingly sparse yield of undiscovered tuberculosis. Community surveys in widely scattered areas—in Sweden, in New York, and in California—have in recent years disclosed significant tuberculosis in only four of every 10,000 roentgenograms. This low prevalence approximates that of sarcoidosis and raises serious questions regarding the economic feasibility of searching for tuberculosis by this method. For this reason and because of the growing reluctance of many persons to undergo periodic exposures to irradiation with such little likelihood of uncovering tuberculosis, tuberculosis associations have explored the possibility of utilizing the tuberculin test as a screening step in case-finding surveys.

The difficulties inherent in this approach are well illustrated by a report of a pilot study conducted in a South Bend, Indiana, hospital in 1957. Custer's report[3] of this attempt to tuberculin test all admissions to a general hospital during a six-month period may be summarized as follows:

3,800 adult patients were admitted to the hospital
2,700 received the tuberculin test (5TU)
2,000 tests were read in the hospital
450 tests were completed by nurses' visits to the home
24.3 per cent of completed tests were positive.

The percentage positive ranged from 19 per cent of those aged twenty-five to thirty-four to 57 per cent of those aged sixty-five to seventy-four.

Roentgenographic examinations were obtained in 500 of the 595 reactors. Not one case of active tuberculosis was found.

It is evident that, despite the extraordinary efforts expended in performing and reading these tests, only 60 per cent of the patients admitted had tests completed. A further large loss ensued in obtaining chest films of the reactors. The failure to find any active tuberculosis raises serious questions as to the practical value of this method of case finding. The yield of tuberculin reactors and of active tuberculosis may be slightly higher in metropolitan hospitals, but what is true of South Bend, Indiana, today will be true throughout the United States in a very few years.

The results of treatment before the development of tuberculosis chemotherapy were so disappointing that tuberculosis physicians seemed unable to face the facts. At any rate, it was not until recently that reports began to appear which accurately reflected the gloomy state of affairs which had existed earlier. DeFriez and his associates[4] in 1954 reported the results of prolonged

[2] *a*. PALMER, C. E., *et al.*: Am. Rev. Tuberc., 1957, *76*, 517. *b*. PALMER, C. E., *et al.*: Am. Rev. Tuberc., 1958, *77*, 877.

[3] CUSTER, E. W.: Am. Rev. Tuberc., 1958, *79*, 378.

[4] DEFRIEZ, A. I., *et al.*: New England J. Med., 1954, *250*, 39.

follow-up study of 377 patients treated by strict bed rest in a Massachusetts sanatorium. There was a cumulative relapse rate at twenty years of 50.1 per cent. Seventy-five per cent of the relapses occurred in the first five years after treatment.

There is good evidence that patients whose disease was arrested by means of surgery and by the use of streptomycin and PAS had considerably lower relapse rates, but it has become clearer each year that extremely low relapse rates are obtained in patients treated with isoniazid. Low[5] has estimated that the total incidence of relapse after present-day chemotherapy will not be more than 1 to 2 per cent.

The role of surgery is steadily diminishing. Surgery is regarded as indicated for only a minority; those patients with persistent cavitary disease, whether they are discharging tubercle bacilli or not, are the only candidates for resection at the present time, and the number requiring such attention grows steadily smaller. It should be emphasized that most of the surgery now being performed in tuberculosis hospitals is prophylactic and is being performed on the hypothesis that the frequency of late recurrences will be less after surgery than after prolonged chemotherapy.

The percentage of newly discovered patients with tuberculosis for whom hospital or sanatorium care is regarded as necessary for the institution of antimicrobial therapy has declined since the introduction of isoniazid. In a discussion of this point at a recent international symposium,[6] physicians accustomed to treating private patients estimated that 50 per cent (Boston), 67 per cent (London), and 80 to 90 per cent (New York) of patients could be treated entirely at home.

Admission to tuberculosis hospitals and sanatoriums was often regrettably long delayed a decade ago. Now entrance is swift, but discharge is inordinately slow. Patients whose disease has resolved within a few months are often confined to hospitals or sanatoriums for the traditional eight- to twelve-month periods. A number of sanatoriums and hospitals, including one of the most prominent (The Trudeau Sanatorium), have closed, and many of the beds occupied in those remaining are filled only because patients are so long confined.

What then is the future of tuberculosis control activities? What should public health authorities and tuberculosis associations do next? It may be suggested that they vie to achieve within the next few years the target point defined by Holm.[7] The World Health Organization considers that tuberculosis will cease to be a major public health problem when no more than 1 per cent of the children in a community are reactors to tuberculin at fourteen years of age. This goal has already been reached in many counties in the Midwest. It will be achieved within a few years in many other parts of the United States.

It is now clear where the last refuge of tuberculosis will be. Tuberculosis in the future is most likely to occur as the result of breakdown of seemingly inactive disease either in patients who once had clinical tuberculosis and recovered, or in patients with small lesions of questionable clinical significance who would in the past have merely been followed with periodic examinations. Patients who recovered from tuberculosis without benefit of isoniazid medication are more likely to experience relapse than are those who received isoniazid. Hence, Oatway's suggestion[8] that a year of prophylactic isoniazid be given every patient who has not had it previously has great merit. Isoniazid in doses of 100 mg. three times daily for a year is inexpensive and well tolerated. It may be feared that patients whose disease has seemingly long been inactive will be greatly disturbed at the suggestion of prolonged medication. On the contrary, patients appear delighted at the opportunity of effectively attacking the tubercle bacillus.

It is estimated by the Public Health Service[9] that in 1956 in the continental United States 550,000 inactive cases of tuberculosis were known to state and local health departments, and an additional 1,200,000 persons had tuberculous lesions of doubtful clinical significance. A majority have not been treated with isoniazid and represent a large reservoir of cases susceptible of reactivation.

A vigorous campaign to give prophylactic isoniazid to these two groups would provide a dramatic end to the antituberculosis crusade. It may be objected that an effort of this magnitude should not be undertaken until proof of its effectiveness is established. To obtain convincing evidence will take many years, during which antituberculosis programs will fizzle out and the opportunity for a telling blow will have been lost.

[5] Low, E.: Am. Rev. Tuberc., 1959, *79*, 612.

[6] International Symposium of the Deborah Sanatorium and Hospital: Am. Rev. Resp. Dis., 1959, *80* (Supplement, p. 118).

[7] International Symposium of the Deborah Sanatorium and Hospital: Am. Rev. Resp. Dis., 1959, *80* (Supplement, p. 42).

[8] Oatway, W. H., Jr.: Am. Rev. Resp. Dis., 1959, *80*, 108.

[9] Glaser, S., *et al.*: Pub. Health Rep., 1957, *72*, 963.

The evidence now at hand appears to many physicians sufficient to justify the extension of isoniazid prophylaxis to all persons with "inactive" tuberculosis who have not previously received this medication.

It might be predicted that the salutary effect will not be confined to the recipients of the isoniazid; an intensive two- or three-year effort directed at cleaning out the "inactive" reservoir will provide many tuberculosis workers with a satisfying conclusion to their efforts in this field.

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Notes

TUBERCULOUS PERICARDITIS

Observed from the Stage of Effusion to Pericardial Calcification

In 1949,[1] the case of an 18-year-old male with acute pericardial effusion caused by tubercle bacilli was reported in this journal. The patient recovered, and it has been possible to examine him periodically since that time. Consequently, the entire period of observation to date is now twenty-two years. As the chest films twenty-two years after the acute infection revealed the development of calcification, it was thought worth while to present a brief report of these follow-up observations at this time.

The details of the initial illness are set forth in the previous publication. In brief, the patient was an 18-year-old male who was admitted to the hospital in July, 1936, with an acute pericardial effusion (figure 1). After a three-month hospital stay that included numerous pericardial taps, the patient was discharged. Tubercle bacilli were demonstrated to be present in the pericardial fluid by the inoculation of guinea pigs.

During the first two years after hospital discharge, the patient led a life of greatly limited activity at home. Beginning in October, 1938, however, he returned to school, from which he was graduated in 1942. Chest films obtained in 1943, 1944, and 1947 showed a gradual reduction in the size of the cardiac shadow (figures 2–4). Additional films were obtained in 1952 (figure 5) and in 1958 (figure 6). The last-named film represents a period of twenty-two years since the acute tuberculous pericardial effusion.

Since the time of the patient's return to school in 1938, he has enjoyed good health and has lost no time from school—or, subsequently, from work—because of illness.

At no time was any cardiac valvular lesion—mitral or aortic—found.

The calcification was first noted in September, 1958. As it is a convex linear calcification along the left border of the ventricle and may be seen on both the postero-anterior and lateral films, it is believed to represent a calcification of the pericardium.

In summary: A twenty-two-year follow-up examination, including roentgenography, is presented of a man who was originally seen with bacteriologically proved tuberculous effusion. During the twenty-two-year follow-up period, the patient has recovered good health and has been essentially asymptomatic. During the last six years of this period, a linear calcification has appeared that presumably represents the development of a calcified pericardium at the site of the old tuberculous process.

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January 5, 1959

[1] KREININ, S., AND COOLEY J.: Am. Rev. Tuberc., 1949, *59*, 650.

(Figures 1–6 appear on following pages.)

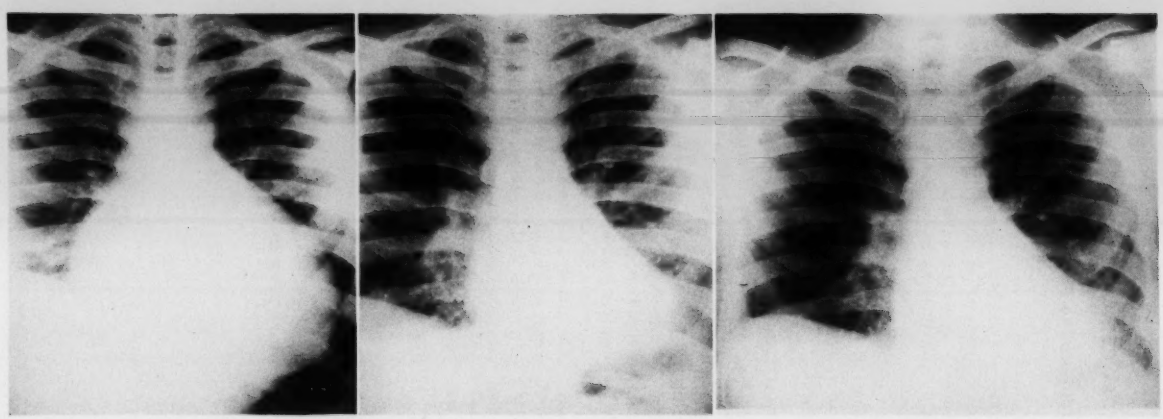

FIG. 1 (Left). Chest roentgenogram obtained on August 3, 1936. The cardiac shadow is markedly enlarged in all directions. It is rounded, it has lost its normal border contours, and has a configuration characteristic of pericardial effusion. There also is an effusion in the right pleural space, fluid reaching up to the level of the fourth rib and axilla, separating the parietal and visceral pleurae and extending into the interlobar fissure between the right lower and middle lobes. The outer third of the right diaphragm is elevated and fixed.

FIG. 2 (Center). Chest roentgenogram obtained on July 10, 1943. The heart is moderately enlarged in all diameters. There is an accentuation of the left and right auricular, as well as the left ventricular, outlines. The findings are those of enlarged heart with possible combined mitral and aortic valvular disease and bilateral diaphragmatic plastic pleurisy. The heart lesion is evidently the residuum of a pancarditis. There is no evidence of pericarditis at this time. A review of a film study made on August 21, 1940, reveals no change.

FIG. 3 (Right). Chest roentgenogram obtained on October 7, 1944. The cardiac contour has decreased greatly in size. The protrusion of the right cardiac border is gone. The right cardiac contour is normal. The left cardiac border still shows some elongation, as by a fixed pericardiopleural or mediastinopleural adhesive pleurisy, but the total size of the heart shadow has returned to approximately normal. The right diaphragm remains elevated and fixed in its outer margin. The right parietovisceral pleurae are separated and thickened. Some fibroproliferative changes are now evident in the left lower lung field.

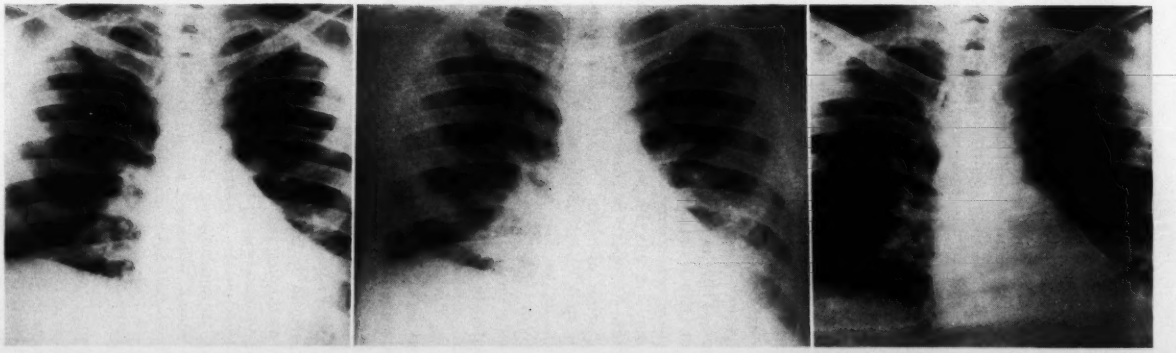

FIG. 4 (Left). Chest roentgenogram obtained on December 1, 1947. The cardiac shadow shows elongation of its left border in the oblique projections. There is an obliterative adhesive pleurisy involving the right diaphragm and the right axillary area. The parietopleural space in each axilla has been obliterated. The right diaphragm is elevated and fixed in its outer third. The left diaphragm is also partially fixed. There are dense basilar mediastinal pleural adhesions and there is a thickening and irregular calcification of the pleura posterolaterally in the lower third of the left chest. The parietovisceral space in the left chest has been obliterated by thickening of the pleura. In view of the history of a previous tuberculous pleurisy, mediastinitis, and pericarditis, these diffuse thickenings of the pleura probably represent the obliterative end stage of a tuberculous pleurisy. The calcific deposit within the pleura further bears this out. Judging by the present findings, the process has involved the pleural areas in each hemithorax, the mediastinal pleura, and the pericardial pleura. There is no evidence of parenchymal tuberculous deposit other than an occasional small, well-calcified primary focus.

FIG. 5 (Center). Chest roentgenogram obtained on January 7, 1952. Roentgenographic examination of the chest shows the right diaphragm to be moderately elevated. The right costophrenic sinus is obliterated by pleural adhesions. There is an area of bronchiole infiltration at both bases, more evident on the right side. The character of the infiltration is strongly suggestive of bronchiectasis. There are gland shadows in each lung root, probably secondary to a basilar infectious process. There is no evidence of tuberculous infection.

FIG. 6 (Right). Chest roentgenogram obtained on September 20, 1958. Roentgenographic examination of the chest reveals evidence of dense calcified and productive deposits in the left lower lung field, as well as clouding of the left costal sinus and left axillary border, indicative of old thickened and calcified pleura. Thickening of the interlobar and marginal pleurae on this side is also noted. A convex linear calcification along the left border of the ventricle is also observed on the postero-anterior and lateral films, suggestive of pericardial calcification. The extent of the linear convexity would favor pericardial calcification rather than interlobar or marginal calcification. A very dense circular band of calcification near the left diaphragmatic area is also seen. Thickening of the axillary and interlobar fissures on the right side can be seen. No recent infiltration can be visualized.

A COMPARISON BETWEEN THE BEHAVIOR OF ESKIMOS AND NON-ESKIMOS TO THE ADMINISTRATION OF ISONIAZID[1]

Middlebrook and Mandel and their associates[2, 3] have reported a simple microbiologic procedure for determining isoniazid concentrations in serum and have presented data indicating that some persons, although receiving doses of the drug generally considered adequate for the treatment of tuberculosis, could not maintain satisfactory concentrations in the tissues. These reports prompted an investigation of the amounts of isoniazid attained in the blood of patients at the Mountain Sanatorium.

In recent years, this institution has been receiving many tuberculous Eskimos from the east coast of the Hudson and James Bay areas, Baffin Island, from settlements along the south shore of Hudson Straits, and from the Ungava Bay area. Experience over the past five years with a total of 835 of these tuberculous Eskimos has indicated that, as a group, they seem to respond to antimicrobial therapy as well as do the tuberculous members of the non-Eskimo population. It was surprising, therefore, to find that 95 per cent of the Eskimo group had almost no "biologically active" isoniazid in the serum six hours after a single 4-mg. per kg. dose of the drug. Moreover, there was good evidence that the remaining 5 per cent were not of pure Eskimo stock. It was demonstrated that 44 per cent of the non-Eskimos in the institution were also "rapid inactivators" of isoniazid, i.e., had 0.6γ or less in the serum drawn six hours after the dosage to which reference has been made. The present study represents an attempt to determine whether any differences are demonstrable in absorption and tissue distribution, excretion, or metabolism between Eskimos and non-Eskimos, particularly rapid inactivator non-Eskimos. It is believed that the results show the only dissimilarity to exist in rate of metabolism: most Eskimos metabolize the drug very rapidly indeed.

In a preliminary experiment, assays were made on single blood specimens taken six hours after isoniazid administration to 500 patients of various racial origins. Most of the non-Eskimos were Canadians by birth or recent immigrants from European countries. All medication was withheld on the day prior to the test except for isoniazid,[4] which was administered in two doses of 8 mg. per kg., one dose prior to 10 a.m. and a second prior to 6 p.m.[5] All other medication was also withheld on the day of the test, when 4 mg. per kg. of isoniazid were given between 9 and 10 a.m. A venous blood specimen was withdrawn exactly six hours later. The serum was removed and kept frozen until examined. The results are summarized in table 1. They show the striking disproportion with regard to rapid inactivators in the Eskimo and non-Eskimo groups.

An effort was made to find out whether there was evidence that the 5 per cent of "slow inactivator" Eskimos might be of mixed racial origin. There is no direct way of determining this other than by studying the physical appearance, as there are no records. With regard to physiognomy, it can be said that one patient had definite European features, and another had some suggestion of them. Blood group studies were made in 3 cases[6] and it was found that the Rh typing in one case would fit the genotype "CDe.-cde" or "Cde.cDe." The research laboratory making the tests stated that "cDe" had not been isolated in an Eskimo population, while "cde" is definitely non-Eskimo. In another case the reaction Le(a+) was found, and this is again more likely to be due to a non-Eskimo gene than to an Eskimo gene. The third case had the reaction Fy(a+b+), and this is exceedingly rare in Eskimos of the Eastern Arctic.

At the time these trials were being conducted, the article by Bell and Riemensnider[7] concerning the metabolic pattern of isoniazid following its oral administration had not yet appeared. In this publication the writers incidentally pointed out that, in determining the amount of isoniazid in the blood, breakfast should be delayed for one and one-half hours after the test dose. No mention

[1] The expenses of this study were defrayed, in large part, by a research grant from the Department of National Health and Welfare, Canada.

[2] Middlebrook, G., *et al.*: "Bacteriologic and Biochemical Aspects of the Chemotherapy of Tuberculosis," exhibit and brochure presented at the annual meeting of the American Trudeau Society, New York, May 20–25, 1956.

[3] Mandel, W., *et al.*: Proc. Soc. Exper. Biol. & Med., 1956, *91*, 409.

[4] Fifty-milligram Isozide tablets, Winley Morris Co.

[5] This preliminary isoniazid "loading" is employed at the National Jewish Hospital at Denver, Colorado (personal communication from Dr. M. L. Cohn).

[6] Tests were kindly performed by Dr. Bruce Chown, Rh Laboratory, Winnipeg, Canada.

[7] Bell, J. C., and Riemensnider, D. K.: Am. Rev. Tuberc., 1957, *75*, 995.

of the need to perform tests in the fasting state had been made in the brochure by Middlebrook and co-workers describing their bioassay, and so this precaution was not observed. Because of the possibility that interference with absorption of isoniazid by food had been a factor in causing the different proportion of rapid inactivators in Eskimos and non-Eskimos, some experiments were done to determine whether this could have been the case. The results of these tests are shown in table 2. The first 17 cases referred to in the table are Eskimos, the last 10 non-Eskimos. The figures suggest that the conclusions would have remained essentially the same had breakfast been withheld. The Eskimos would still have been nearly all rapid inactivators.

More detailed studies were made on a group of 25 Eskimos and an equal number of non-Eskimos. Twenty-three of the former and 11 of the latter were rapid inactivators, reflecting the proportions found in the respective ethnic groups as a whole. The plan was to investigate the concentration of isoniazid in serum over an entire absorption period in these patients, complemented by quantitative assessments of the output of free drug in the urine. Since the number of blood specimens which can fairly be taken from patients for experimental purposes is limited, however, and since the entire absorption period—so far as measurable amounts of isoniazid in the blood is concerned—is quite long in slow inactivators (eighteen hours or more), sampling times had to be selected which were a compromise. For slow inactivators, three, six, nine, and twelve hours were selected, while for rapid inactivators, one, three, and six hours seemed the most informative. Urine specimens were collected at two-hour intervals up to twelve hours, and then for the second twelve-hour period the output was pooled to make a single specimen.

On the day prior to the test, all medication was withheld, including isoniazid. Thus, there was no preliminary "loading" dose as in the previously described tests. On the day of the test, 4 mg. per kg. of isoniazid were administered orally and breakfast was withheld for one and one-half hours.

The results of the studies are shown in table 3, in which the rapid inactivators have been separated from the slow inactivators. In each of these two divisions the Eskimos have been separated from the non-Eskimos, and these racial groups then arranged in order of increasing amounts of "biologically active" isoniazid in blood withdrawn three hours after the test dose (because at six hours the method is not sensitive enough to differentiate degrees of inactivation in many cases). Taking into account the error of the method (± 20 per cent), no significant difference between the two groups of rapid inactivators is apparent, except that very low values for isoniazid are present in a number of Eskimo blood

TABLE 1

ANALYSIS OF DETERMINATIONS OF BIOLOGICALLY ACTIVE ISONIAZID IN SERUM FROM 500 SANATORIUM PATIENTS

All cases			
Rapid inactivators	(0.6 γ per ml. or less)	331	(66%)
Slow inactivators	(0.8 γ per ml. or more)	169	(34%)
Non-Eskimos			
Rapid inactivators	(0.6 γ per ml. or less)	125	(44%)
Slow inactivators	(0.8 γ per ml. or more)	159	(56%)
Eskimos			
Rapid inactivators	(0.6 γ per ml. or less)	206	(95%)
Slow inactivators	(0.8 γ per ml. or more)	10	(5%)

TABLE 2

INFLUENCE OF FOOD ON THE AMOUNT OF BIOLOGICALLY ACTIVE ISONIAZID IN THE SERUM SIX HOURS AFTER A TEST DOSE OF 4 MG. PER KG.

Case Number	Fasting	Breakfast	Case Number	Fasting	Breakfast	Case Number	Fasting	Breakfast
	(γ per ml.)			(γ per ml.)			(γ per ml.)	
1	0.2	<0.2	10	<0.2	<0.2	19	3.5	4.8
2	<0.2	<0.2	11	<0.2	<0.2	20	0.8	0.8
3	<0.2	<0.2	12	<0.2	0.2	21	3.2	4.6
4	<0.2	0.2	13	<0.2	<0.2	22	3.2	6.0
5	<0.2	0.2	14	<0.2	<0.2	23	0.4	0.6
6	0.2	<0.2	15	0.4	<0.2	24	3.5	3.7
7	<0.2	<0.2	16	1.6	<0.2	25	0.4	0.4
8	<0.2	<0.2	17	0.4	0.6	26	3.5	3.5
9	<0.2	<0.2	18	0.4	0.4	27	0.8	0.8

TABLE 3

BIOLOGICALLY ACTIVE ISONIAZID (IN γ PER ML.) IN SERUM SPECIMENS OF ESKIMOS AND NON-ESKIMOS AT VARIOUS INTERVALS AFTER A SINGLE 4-MG. PER KG. DOSE

		Case Number	1 Hour	3 Hours	6 Hours	9 Hours	12 Hours
Rapid Inactivators	Eskimos	1	1.6	<0.2	<0.2		
		2	1.6	<0.2	<0.2		
		3	1.6	<0.2	<0.2		
		4	3.2	<0.2	<0.2		
		5	4.0	<0.2	<0.2		
		6	1.2	0.2	<0.2		
		7	0.6	0.4	<0.2		
		8	1.2	0.4	<0.2		
		9	4.0	0.4	<0.2		
		10	4.0	0.4	<0.2		
		11	4.0	0.4	<0.2		
		12	1.6	0.6	<0.2		
		13	5.6	0.6	<0.2		
		14	5.6	0.6	<0.2		
		15	5.6	0.6	<0.2		
		16	5.6	0.6	<0.2		
		17	7.2	0.6	<0.2		
		18	7.2	0.6	<0.2		
		19	5.6	0.8	<0.2		
		20	5.6	0.8	0.2		
		21	7.2	0.8	<0.2		
		22	8.8	1.2	0.2		
		23	7.2	1.6	<0.2		
	Non-Eskimos	24	1.6	0.6	<0.2		
		25	4.0	0.6	<0.2		
		26	7.2	0.6	<0.2		
		27	3.2	0.8	<0.2		
		28	4.0	0.8	<0.2		
		29	7.2	0.8	<0.2		
		30	5.6	1.2	<0.2		
		31	7.2	1.2	<0.2		
		32	8.8	1.2	<0.2		
		33	8.8	1.6	<0.2		
		34	8.8	1.6	0.2		
Slow Inactivators	Eskimos	35		5.6	3.6	1.2	0.6
		36		7.2	5.2	1.2	0.6
	Non-Eskimos	37		4.0	1.2	0.6	0.4
		38		5.6	1.6	0.8	0.4
		39		5.6	1.6	1.2	0.4
		40		5.6	4.0	1.6	0.8
		41		6.4	1.6	0.6	0.4
		42		6.4	3.2	0.8	0.8
		43		6.4	3.6	0.8	0.6
		44		6.4	3.6	0.8	0.6
		45		6.4	4.0	1.2	0.6
		46		6.4	4.0	1.2	0.8
		47		6.4	4.8	1.6	1.2
		48		7.2	1.6	1.2	0.6
		49		7.2	3.2	0.8	0.6
		50		7.2	4.0	1.2	0.6

specimens at three hours which are not matched by any in the non-Eskimo group. This may be simply a reflection of the small numbers studied; but it is more probable that the degree of inactivation tends to be greater in a given length of time in Eskimos, as will be pointed out later. Judging by the blood level attained in one hour, absorption from the gastrointestinal tract is evidently rapid and of about the same order in the early stages in both rapid inactivator Eskimos and non-Eskimos. Although there are only 2 Eskimos among the slow inactivators studied, nevertheless it can be seen that each of the sets of values for them can be very nearly matched with one or more of those for their non-Eskimo counterparts.

The experiments just described were supplemented by others in which 16 mg. per kg. of isoniazid were given to Eskimos in two 8-mg. per kg. doses on the day prior to the test dose of 4 mg. per kg., and in these experiments the blood specimens were obtained at one, two, four, and six hours. The pattern was the same and, in addition, it was noted that at two hours the serum values had, in most Eskimos, already begun to fall.

The urine assays on the 50 patients were performed on the same occasion as the blood studies and were done by the method of Maher and associates[8] which, in contrast to the biologic method necessarily used for blood studies because of the minute quantities present, is capable of a high degree of accuracy. The results are recorded in table 4, which shows the values at two-hour intervals, and in table 5, which summarizes this and also records the findings for the second twelve hours as well as the recovery of administered isoniazid. The cases are arranged in the same order as in table 3.

The amounts excreted during any given interval are very variable from one individual to another and, because of this, it is obvious that, to detect small differences between one group of people and another, a much larger series of cases would have to be studied. But it was believed that, if there was any real dissimilarity between the Eskimo and non-Eskimo metabolism of isoniazid, some definite trend ought to be evident with the data available. This was not the case. The data do show that, following administration, the tendency is for a peak to be reached for total excretion in the two- to four-hour period; after this there is a slow fall, so that by the end of twelve hours an average of 82 per cent of what is going to be excreted renally is already eliminated. Approximately 18 per cent more is excreted in the next

[8] MAHER, J. R., *et al.*: Am. Rev. Tuberc., 1957, *76*, 852.

TABLE 4

COMPARISON OF THE URINARY EXCRETION OF TOTAL ISONIAZID AND ACTIVE ISONIAZID* IN ESKIMOS AND NON-ESKIMOS AFTER A 4-MG. PER KG. DOSE OF ISONIAZID

		Case Number	Total Isoniazid in Milligrams in Two-Hour Periods						Active* Isoniazid in Milligrams in Two-Hour Periods						Per Cent of Total Isoniazid Excreted in the Active* Form, in Two-Hour Periods					
			0–2	2–4	4–6	6–8	8–10	10–12	0–2	2–4	4–6	6–8	8–10	10–12	0–2	2–4	4–6	6–8	8–10	10–12
Rapid Inactivators	Eskimos	1	53.0	50.0	24.0	3.2	13.6	3.3	11.3	6.0	2.4	0.3	1.6	0.8	21	12	10	9	12	24
		2	46.0	49.0	70.0	32.2	23.7	15.4	5.1	4.4	2.3	0.8	0.9	0.2	11	9	3	2	4	1
		3	28.6	35.5	39.2	12.1	18.2	14.8	7.4	4.2	3.5	0.5	2.6	0.6	26	12	9	4	14	4
		4	38.6	42.0	28.0	18.8	17.9	13.7	7.4	0.9	2.4	1.3	3.3	0.7	19	2	9	7	18	5
		5	—	39.5	59.5	8.6	21.8	10.1	—	2.0	3.8	0.4	1.5	0.3	—	5	6	5	7	3
		6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		7	14.5	18.1	14.6	1.8	7.3	5.5	9.0	3.8	2.7	0.5	2.5	0.7	62	21	19	28	34	13
		8	35.0	61.5	42.0	28.0	22.0	13.0	6.4	15.5	9.5	4.4	2.4	1.0	18	25	23	16	11	7
		9	43.2	30.0	30.8	23.2	23.8	25.8	8.7	6.0	2.3	7.7	5.5	3.8	20	20	7	33	23	15
		10	56.0	35.0	19.0	56.0	23.5	9.0	7.0	1.6	0.2	1.5	1.1	0.2	12	5	1	3	5	2
		11	50.2	32.7	10.9	26.6	5.1	9.3	7.4	3.8	0.7	5.9	1.5	2.0	15	12	6	22	29	21
		12	15.0	37.0	5.1	2.5	14.8	9.0	4.0	4.3	0.8	0.5	1.8	0.6	27	12	16	20	12	7
		13	0.4	81.0	34.4	24.2	15.2	11.7	0.3	17.1	4.1	0.8	4.0	4.1	75	21	12	3	26	35
		14	39.6	52.8	27.0	14.5	14.8	7.7	10.4	9.8	2.6	0.4	1.2	0.2	26	19	10	3	8	3
		15	21.2	42.1	35.8	26.2	16.6	12.8	7.7	10.1	4.2	2.9	1.6	0.6	36	24	12	11	10	5
		16	40.8	48.5	33.0	20.3	26.2	17.4	11.2	4.8	1.6	3.8	0.8	0.7	27	10	5	19	3	4
		17	35.0	53.5	36.5	22.6	16.0	13.2	8.4	8.5	2.7	0.2	0.8	0.7	24	16	7	1	5	5
		18	35.0	17.2	13.8	17.8	17.1	4.7	9.7	2.4	1.5	0.5	3.0	0.7	28	14	11	3	18	15
		19	25.3	52.3	31.5	26.6	11.8	10.4	11.2	14.3	2.1	2.4	0.8	1.3	44	27	7	9	7	12
		20	39.8	38.6	33.4	21.3	17.8	14.6	13.2	12.0	4.0	1.5	0.8	0.9	33	31	12	7	5	6
		21	25.0	30.5	10.6	13.4	10.2	9.4	8.6	5.8	0.4	2.6	0.8	0.8	34	19	4	19	8	9
		22	21.7	31.7	45.0	32.8	18.8	14.0	7.0	8.3	4.4	1.9	0.8	1.8	32	26	10	6	4	13
		23	16.6	20.9	18.1	30.1	17.2	6.8	5.0	7.4	2.3	1.9	0.4	0.0	30	35	13	6	2	0
	Non-Eskimos	24	45.3	36.5	38.2	36.2	26.3	16.9	7.9	4.5	1.8	0.3	0.6	0.2	17	12	5	1	2	1
		25	18.5	37.0	16.2	—	17.6	13.4	6.8	7.1	1.9	—	1.9	2.2	37	19	12	—	11	16
		26	49.0	34.0	28.5	23.7	13.1	9.0	12.2	4.1	1.5	0.6	0.4	0.2	25	12	5	3	3	2
		27	43.0	65.0	32.0	32.0	20.0	19.0	7.6	16.2	1.4	1.1	0.8	0.7	18	25	4	3	4	4
		28	24.3	32.0	22.8	14.3	10.9	9.6	5.2	6.7	1.7	1.6	0.8	0.3	21	21	7	11	7	3
		29	25.8	28.7	21.6	26.2	13.6	9.2	8.0	4.9	2.8	1.3	0.9	0.5	31	17	11	5	7	5
		30	17.8	54.0	30.8	22.3	17.1	10.6	6.1	8.9	2.5	1.7	3.0	0.9	34	16	8	8	18	8
		31	31.0	31.8	18.0	6.2	11.2	14.8	11.8	8.2	3.8	0.9	1.7	1.6	38	26	21	15	15	11
		32	—	53.9	5.9	36.0	29.2	20.8	—	10.9	0.75	3.6	1.6	1.5	—	20	13	10	5	7
		33	20.5	8.8	14.8	16.2	20.3	11.3	7.9	3.9	5.3	1.8	0.8	0.4	39	44	36	11	4	4
		34	31.4	33.8	36.4	26.5	17.4	13.8	8.3	4.9	2.0	1.0	0.5	0.2	26	15	6	4	3	1
Slow Inactivators	Eskimos	35	27.0	40.0	20.0	15.0	9.0	9.0	22.5	29.4	10.8	7.2	7.8	4.3	83	74	54	48	87	48
		36	24.8	32.8	32.9	23.7	22.0	12.8	16.2	22.0	18.4	11.4	9.2	4.8	65	67	56	48	42	38
	Non-Eskimos	37	16.4	27.2	13.1	4.5	13.4	5.8	7.4	13.2	5.1	1.1	4.0	0.5	45	49	39	24	30	9
		38	17.2	47.0	20.0	22.0	18.5	13.6	15.2	25.0	7.5	4.8	4.2	4.0	88	53	38	22	23	29
		39	32.7	49.5	25.5	29.8	16.0	12.9	21.2	29.3	9.4	12.3	4.6	3.9	65	59	37	41	29	30
		40	12.2	30.3	22.5	19.2	17.3	13.6	8.1	20.7	13.8	10.5	7.5	5.9	66	68	61	55	43	43
		41	43.5	31.2	30.0	27.5	21.2	14.9	23.8	14.1	18.8	14.1	10.8	12.4	55	45	63	51	51	83
		42	19.3	28.4	22.5	7.9	11.1	4.2	12.6	15.0	7.2	2.9	3.4	0.7	65	53	32	37	31	17
		43	18.2	22.8	21.4	6.5	13.1	8.9	9.9	10.1	9.6	2.8	1.3	0.2	88	44	45	45	10	2
		44	5.2	20.5	19.2	19.8	14.3	12.1	3.4	12.3	10.0	7.6	5.5	5.3	65	60	52	38	38	44
		45	24.4	34.5	28.4	29.4	16.0	12.4	18.4	25.5	16.4	16.2	7.2	5.5	75	74	58	55	45	44
		46	16.9	32.3	12.3	36.5	20.7	23.6	9.4	18.8	5.9	14.6	6.6	7.6	56	58	48	40	32	32
		47	11.5	30.6	23.8	34.0	6.9	44.8	7.3	20.8	13.5	20.1	6.2	11.1	63	68	57	59	90	25
		48	33.0	39.5	30.5	27.6	20.3	18.2	23.0	23.3	14.3	10.4	6.1	5.8	70	59	47	38	30	32
		49	24.4	42.0	32.0	16.8	19.6	14.9	16.0	26.5	16.2	9.8	6.1	4.7	66	63	51	58	31	31
		50	22.5	35.8	28.3	15.8	14.6	16.9	15.0	25.5	15.7	8.4	0.9	6.1	67	71	55	53	6	36

* Free isoniazid plus pyruvic acid and alpha-ketoglutaric acid isonicotinoyl hydrazones.

TABLE 5

Comparison of Urinary Excretion of Total Isoniazid and Active Isoniazid* in Eskimos and Non-Eskimos after a 4 Mg. per Kg. Dose of Isoniazid

		Case Number	Total Isoniazid in Milligrams in 12-Hour Periods		Active* Isoniazid in Milligrams in 12-Hour Periods		Per Cent of Total Isoniazid Excreted in the Active* Form, in 12-Hour Periods		Per Cent Recovered in 24 Hours
			0–12	12–24	0–12	12–24	0–12	12–24	
Rapid Inactivators	Eskimos	1	147.1	6.8	22.4	1.6	15	24	87
		2	236.3	25.6	13.7	1.8	6	7	104
		3	148.4	24.1	18.3	1.7	13	7	86
		4	159.0	26.3	16.0	1.2	10	5	82
		5	139.0†	25.2	8.8†	0.8	6	3	60‡
		6	—	—	—	—	—	—	—
		7	61.8	8.7	19.2	1.6	31	18	71
		8	201.5	26.0	39.2	1.9	19	7	101
		9	176.8	52.6	34.0	3.6	21	7	106
		10	198.5	26.5	11.6	1.1	6	4	90
		11	134.8	12.7	21.3	2.7	16	21	65
		12	83.4	24.0	12.0	7.6	14	32	54
		13	166.9	17.3	30.4	4.2	18	24	82
		14	156.4	14.7	24.6	1.8	16	12	98
		15	154.7	20.8	27.1	2.0	17	10	77
		16	156.5	19.5	22.9	3.5	15	18	82
		17	176.8	23.3	21.3	1.4	12	6	88
		18	105.6	14.3	17.8	5.0	17	35	60
		19	157.9	27.8	32.1	16.7	20	60	92
		20	165.5	38.3	32.4	3.1	20	8	81
		21	99.1	10.5	19.0	0.7	19	7	62
		22	164.0	45.5	24.2	11.7	15	26	70
		23	109.7	26.5	17.0	1.0	16	4	61
	Non-Eskimos	24	199.4	42.3	15.4	5.4	8	13	69
		25	102.7	26.2	19.9	0.7	19	3	69
		26	157.3	20.0	19.0	0.4	12	2	78
		27	211.0	46.2	27.8	7.5	13	16	93
		28	113.9	26.2	16.3	4.4	14	17	93
		29	125.1	28.8	18.4	1.5	15	5	77
		30	152.6	19.6	23.1	2.0	15	10	76
		31	113.0	31.5	28.0	1.4	25	4	58
		32	145.8†	49.2	8.3†	1.2	13†	2	53‡
Slow Inactivators	Eskimos	33	91.9	55.0	20.1	2.1	22	4	42
		34	159.3	24.0	16.9	0.8	11	3	81
		35	120.0	18.0	82.0	0.2	68	67	55
		36	149.0	26.5	82.0	6.8	55	26	63
	Non-Eskimos	37	80.4	27.7	31.3	6.5	39	23	48
		38	138.3	22.5	60.7	3.4	44	15	70
		39	166.4	32.7	80.7	8.6	48	26	66
		40	115.1	34.0	66.5	10.3	58	30	60
		41	168.3	44.6	94.0	4.1	56	39	77
		42	93.4	17.5	41.8	6.4	45	38	44
		43	90.9	35.9	33.9	10.0	44	28	46
		44	91.1	31.5	44.1	7.0	48	22	70
		45	145.1	41.8	89.2	13.4	61	32	68
		46	142.3	43.0	62.9	10.8	44	25	62
		47	151.6	49.6	79.0	16.1	52	32	67
		48	169.1	39.0	82.9	6.3	49	16	75
		49	149.7	33.8	79.3	5.8	53	17	66
		50	133.9	38.8	71.6	9.7	54	25	69

* Free isoniazid plus pyruvic and alpha-ketoglutaric isonicotinoyl hydrazones.

† 2–12 hours only.

‡ 2–24 hours only.

TABLE 6

Comparison of Biologically Active Isoniazid in Serum of "Rapid Inactivator" Eskimos and Non-Eskimos Six Hours after Administration of Various Doses of Isoniazid, and after Isoniazid and PAS

	Biologically Active Isoniazid in Serum in γ per Ml.			
	After 4 Mg. per Kg. of Isoniazid	After 8 Mg. per Kg. of Isoniazid	After 16 Mg. per Kg. of Isoniazid	After 4 Mg. per Kg. of Isoniazid and 21 Gm. of Sodium PAS
Eskimos	<0.2	<0.2	0.8	<0.2
	<0.2	<0.2	0.8	<0.2
	<0.2	<0.2	0.8	<0.2
	<0.2	0.4	1.2	<0.2
	<0.2	<0.2	0.6	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	0.6	<0.2
	<0.2	<0.2	<0.2	<0.2
	<0.2	<0.2	0.4	<0.2
	<0.2	<0.2	0.6	<0.2
Non-Eskimos	0.4	0.8	5.6	0.6
	<0.2	0.8	4.0	0.6
	<0.2	0.4	1.6	0.4
	<0.2	0.6	3.2	0.6
	<0.2	0.2	0.8	<0.2
	<0.2	0.6	1.6	0.4
	<0.2	0.2	3.2	0.6
	<0.2	0.4	4.0	0.6
	<0.2	0.4	1.6	<0.2
	<0.2	0.8	4.0	0.6

TABLE 7

Peroxidase and Catalase Activities and Susceptibility to Isoniazid of Tubercle Bacilli Isolated from Eskimos after Three Months of Treatment with Isoniazid

Name	Peroxidase Activity	Catalase Activity	Susceptibility of Culture to 10 γ of Isoniazid per Ml.
Naksook	+	++	Susceptible
Ooshooweetook	+	++	Susceptible
Mosesie	+	++	Susceptible
Angutadjuak	−	−	Resistant
	++	+	Resistant
Kookee	−	++	Resistant
Fleming	−	++	Resistant
Nikotiak	−	++	Resistant*
	−	++	Resistant
Josie	−	++	Resistant
	−	++	Resistant
	−	−	Resistant
Goota	−	−	Resistant

* The highest concentration of isoniazid tested on this strain was 1.28 γ per ml.

twelve hours. No significant difference can be observed, however, between Eskimos and non-Eskimos. On the average, rapid inactivators excrete only one-third to one-quarter as much isoniazid in the free form as do those classified as slow inactivators. In these particular tests, the former voided urine in which, for a twelve-hour period, active drug was present to the extent of only 6–31 per cent (average 15 per cent) of the total eliminated, as against 39–68 per cent (average 45 per cent) for the latter.[9] The Eskimos behaved in essentially the same way as did the others.

In 3 instances the recovery from Eskimos of the isoniazid administered was slightly beyond 100 per cent. There are two obvious possibilities: *(1)* The twenty-four-hour drug-free period prior to the test may have been too short to prevent some carry-over into the test urine. *(2)* These Eskimos may not have followed instructions accurately with regard to the beginning of the collection period. They spoke little or no English and it was very difficult, for this reason among others, to obtain full cooperation.

The possibility that differences might exist between Eskimos and non-Eskimos in terms of the concentrations of isoniazid attainable in the blood was investigated as follows: Ten known rapid inactivator Eskimos and 10 rapid inactivator non-Eskimos each received 4, 8, and 16 mg. per kg. of isoniazid in a single dose, and six hours later blood was withdrawn for assay. One week was allowed to elapse between each of the three dosage trials, and in the intervening days the patient received either no isoniazid or only the moderate doses being employed at the time for treatment of tuberculosis, such as 4 mg. per kg. On the day prior to each test, no drugs of any kind were given. The same groups of subjects were also used to examine the influence of PAS on the amounts of isoniazid in serum specimens. Again, one week was allowed to elapse between this and any previous test. The results are presented in table 6.

The response to 4 mg. per kg. was similar in all of the patients of both groups except *Case 1* of the non-Eskimos, the exact amount of isoniazid in the serum always being too low to measure with the method employed ($<0.2\gamma$ per ml.). After receiving 8 mg. per kg. there was a detectable increase in isoniazid in the serum in only one Eskimo, whereas all serum specimens from the other group showed higher values. After 16 mg. per kg. there were still 2 serum specimens from Eskimos in which no increase could be detected, while the other specimens from the other Eskimos did show a two- to fourfold increase. On the other hand, all of the non-Eskimo specimens had, after this large dose, from three to sixteen times as much active drug as they did after an 8-mg. per kg. dose.

Administration of PAS was ineffective in producing a demonstrable increase in the amount of "active" isoniazid in the blood of Eskimos, but there was a change in the values in 8 of the 10 non-Eskimo blood specimens. Some additional studies with PAS were made in which patients received two 8-mg. per kg. doses of isoniazid on the day prior to testing. The results were in agreement with those just described. Whereas these findings suggest a slight difference in behavior of the two groups, closer inspection of the table shows that this is not necessarily so, because the method employed does not measure how far below 0.2γ per ml. most of the drug concentrations were after the lowest dose studied. It may be that the Eskimo actually responded very well to increased dosage. This could have been the case if the real value for Eskimos after 4 mg. per kg. had been very much lower than for the non-Eskimos, or even zero.

The premise that, as a group, tuberculous Eskimos respond to antimicrobial therapy as well as other patients, implies in the light of the work herewith reported that a lower demonstrable quantity of isoniazid in serum, and presumably in tissues, suffices to arrest the disease than is the case with non-Eskimos. An effort was made to confirm the truth of the statement by assessing carefully the clinical and roentgenographic progress of the group of patients on whom the blood assays were made. It was found that this was impracticable for a variety of reasons. An attempt was made, therefore, to approach the problem from a bacteriologic point of view; but few patients excrete bacilli long enough during the course of their disease to be of much help. It was possible,

[9] In another series of 50 cases, the figures were very much the same. This lends support to a belief, heard expressed verbally by others, that urinary tests can predict whether a subject will, on the basis of a biologic test with serum, be classed as a rapid or slow inactivator. But it must be pointed out that all of the rapid inactivators in this study happened to be highly so. Even then, if one compares the twelve-hour urine for isoniazid excretion percentages of *Cases 38, 46, and 41* (table 5) with the actual blood serum values six hours after isoniazid administration (table 3), it can be observed that a urine value of 44 per cent active isoniazid in the total excreted can be matched with a serum of 1.6γ per ml. or one of 4.0γ per ml.; and that urine excretion percentages of 44 and 56 per cent both will match serum of 1.6γ per ml. Therefore, whereas urinalyses may predict which persons are likely to be *very* rapid or *very* slow inactivators by the serum microbiologic assay, there must be a borderline group which cannot be categorized by this means.

however, to perform catalase and peroxidase tests on organisms obtained from 9 patients after three months of therapy. The findings are shown in table 7. The excretion of peroxidase- or catalase-positive tubercle bacilli suggests that the amount of drug reaching the organisms had been inadequate either in concentration, time of contact, or both. The results clearly indicate that these particular patients either were not receiving enough isoniazid to influence the bacilli effectively, or the amount given was being spaced out over too much of the day.

Assuming that the balance of the Eskimos really did respond well, it must be that a concentration of isoniazid in blood, and presumably tissues, of about 1γ per ml. for about three hours daily is adequate. As a matter of fact, experiments in this laboratory show that growth of tubercle bacilli *in vitro* can be inhibited completely if for six days the organisms are exposed to a concentration of 1γ per ml. for a period of three hours only, the drug-containing medium being replaced with plain medium for the rest of each day. Details of this particular phase of the study are the subject of another communication,[10] because it appears that precise information on the relationship, *in vitro*, between daily duration of drug contact and effect on bacilli is not presently available.

In summary: Ninety-five per cent of Eskimos have less than 0.6γ per ml. of isoniazid in their serum specimens obtained six hours after a single 4-mg. per kg. oral dose. There is evidence that at least some of the remainder are of mixed racial origin. The absorption and excretion of isoniazid are crudely the same in Eskimos and in the 44 per cent of the non-Eskimo population classed as "rapid inactivators." For example, in the first twelve hours after an oral dose of 4 mg. per kg., 23 rapid inactivator Eskimos excreted 6–31 per cent of the total urinary isoniazid in the free or active form; 11 non-Eskimo rapid inactivators excreted 8–25 per cent in this form.

The only demonstrated difference in behavior to administration of isoniazid between the two groups was a greater rise in the amount of active isoniazid in the serum of non-Eskimos six hours after dosage in response to an increase in the amount of drug given or the concurrent administration of PAS. This difference is probably apparent rather than real, and is related to the fact that the method used in the assay did not permit measurement of amounts less than 0.2γ of isoniazid per ml. of serum.

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June 9, 1959

[10] Armstrong, A. R.: Am. Rev. Resp. Dis., 1960, *81*, 498.

[11] The writers are grateful to Dr. M. L. Cohn for receipt of a culture of the test organism H37RvSR10SM.

THE COMPARATIVE SPECIFICITY BETWEEN LÖWENSTEIN-JENSEN MEDIUM AND A MODIFIED OLEIC-ACID–ALBUMIN MEDIUM IN SUPPORTING THE GROWTH OF TUBERCLE BACILLI

During the period from January, 1958, to July, 1959, all sputum and gastric specimens submitted for examination for *M. tuberculosis* were simultaneously inoculated into two different media: Löwenstein-Jensen medium,[1] and an oleic-acid–albumin medium described by Middlebrook and associates,[2] modified by the Research and Development Service of Fitzsimons Army Hospital, and designated RDM-1.[3] During the eighteen-month period of this study, 645 sputum and gastric specimens were reported positive for *M. tuberculosis* on either of these media or on both. Although each of the 645 specimens was responsible for at least one culture positive for *M. tuberculosis*, a single specimen was frequently responsible for a positive culture on Löwenstein-Jensen medium and a negative culture on the modified oleic-acid–albumin medium. Clinical observations suggested that the incidence of positive cultures obtained from the same sputum or gastric specimens was higher with Löwenstein-Jensen medium than with the modified oleic-acid–albumin medium. This informal impression did not seem to correspond with the experiences of other co-workers who have utilized both of these media. The following report is aimed at an evaluation of the specificity of Löwenstein-Jensen medium versus the modified oleic-acid–albumin medium in their ability to produce cultures positive for *M. tuberculosis* after being simultaneously inoculated with fresh sputum or gastric specimens.

During the eighteen-month period of this study, 645 sputum or gastric specimens obtained from patients with active tuberculosis were reported positive on culture for *M. tuberculosis*. As noted above, each of these specimens was simultaneously inoculated into Löwenstein-Jensen medium (hereafter designated L-J medium), and a modified oleic-acid–albumin medium (hereafter designated RDM-1 medium). Sputum and gastric materials were collected and prepared in the following manner: Specimens were collected in sterile bottles containing approximately 15 ml. of 5.67 per cent trisodium phosphate. This mixture was shaken on a mechanical shaker for fifteen minutes, placed in an incubator at 37°C. for twenty-four hours, and then centrifuged at 2,000 r.p.m. for fifteen minutes. The supernatant liquid was then decanted and an equal amount of Sorenson's buffer (at pH 6.6 with Tween® 80) was added to the precipitate. This mixture was washed by agitation and again centrifuged for fifteen minutes. The supernatant liquid was again decanted and the precipitate was used to inoculate the cultures. One tube of L-J medium was inoculated with 0.5 ml. of this residuum, and two Petri dishes containing RDM-1 medium were simultaneously inoculated with an equal amount of the residuum. The tubes were capped and the Petri dishes were placed in plastic bags. All cultures were then incubated at 37°C. The presence of growth was recorded on the L-J medium after six weeks' incubation, and on the RDM-1 medium after four weeks of incubation.

The 645 sputum and gastric specimens which produced cultures positive for *M. tuberculosis* were obtained from 197 patients. Since each of these specimens was inoculated into one tube of L-J and two Petri dishes of RDM-1 media, a total of 1,935 cultures was reviewed.

Each of the patients whose specimens were examined in this study received one of the three following drug regimens: 73 received 10 to 20 mg. of isoniazid per kg. of body weight and 12 gm. of aminosalicylic acid daily; 65 received streptomycin, 1.0 gm. daily, in addition to high doses of isoniazid with aminosalicylic acid; and 59 received 300 mg. of isoniazid plus 12 gm. of aminosalicylic acid daily. All patients receiving more than 300 mg. of isoniazid daily were also given 100 mg. of pyridoxine. The incidence of cultures positive for *M. tuberculosis* on L-J and RDM-1 media was tabulated for the whole group. In addition, the incidence of positive cultures on each of these media, with relation to the duration of chemotherapy prior to the implantation of cultures, was also tabulated.

In table 1, the results of cultures obtained from 645 sputum and gastric specimens are tabulated. As noted above, each of these 645 specimens produced a culture positive for tubercle bacilli on either the L-J or the RDM-1 medium, or on both of these media. Six hundred and eleven, or 94.7 per cent, of 645 specimens produced positive cultures on L-J medium. Only 379 specimens produced positive cultures on RDM-1 medium. The incidence of positive cultures was, therefore, 35.9 per cent higher on the L-J medium. Only 53.5 per cent of the 645 specimens were simul-

[1] JENSEN, K. A.: Zentralbl. f. Bakt. (Abt I), 1932, *125*, 222.

[2] MIDDLEBROOK, G., *et al.*: Am. Rev. Tuberc., 1954, *70*, 852.

[3] Laboratory Methods for Tuberculosis Bacteriology, Report No. I, 1957, Research and Development Service, Fitzsimons Army Hospital, Denver, Colorado.

TABLE 1

645 Sputum and Gastric Specimens with Cultures Positive for Tubercle Bacilli

	Number Positive	Per Cent Positive
Total number of specimens with positive cultures	645	
Total number of specimens with positive cultures on L-J medium*	611	94.7
Total number of specimens with positive cultures on RDM-1 medium†	379	58.8
Number of specimens positive on both L-J and RDM-1 medium	345	53.5
Number of specimens positive on L-J and negative on RDM-1 medium	266	41.2
Number of specimens positive on RDM-1 and negative on L-J medium	34	5.3

* L-J—Löwenstein-Jensen
† RDM-1—Modified oleic-acid-albumin

TABLE 2

The Influence of Drug Therapy upon the Incidence of Cultures Positive for Tubercle Bacilli

Number of Months of Treatment before Culture was Inoculated	Number of Specimens Positive on Culture	L-J Medium*		RDM-1 Medium†	
		Number Positive	Per Cent Positive	Number Positive	Per Cent Positive
0	423	402	95.0	261	61.7
1	104	95	91.3	61	58.7
2	50	45	90.0	27	54.0
3–5	29	28	96.6	12	41.4
6 or more	39	38	97.4	18	46.2

* L-J—Löwenstein-Jensen
† RDM-1—Modified oleic-acid-albumin

taneously positive on both L-J and RDM-1 media. In 300 of these 645 specimens, only one of the two media used in this study was positive for *M. tuberculosis*. Of the 300 specimens producing single positive cultures, 266 were positive on L-J medium and only 34 were positive on RDM-1 medium. The 266 specimens which produced positive cultures on L-J medium alone represent 41.2 per cent of the total group of 645 specimens; but they also represent 88.7 per cent of the 300 specimens which produced positive cultures on only one of the two media used.

In table 2, the incidence of cultures positive for tubercle bacilli on L-J and RDM-1 media is tabulated with respect to the duration of chemotherapy administered to patients prior to the inoculation of the cultures. Ninety per cent or more of all of the specimens produced positive cultures on L-J medium, regardless of the amount of chemotherapy administered to the patient prior to the inoculation of the culture. The incidence of positive cultures on RDM-1 medium was highest (61.7 per cent) in those patients who had not received any chemotherapy before inoculation of cultures. With the RDM-1 medium, the incidence of positive cultures progressively decreased as the duration of chemotherapy prior to inoculation of cultures increased. After three months of chemotherapy, the incidence of positive cultures on RDM-1 medium was 15 to 20 per cent lower when compared with the rate of positive cultures (on RDM-1 medium) obtained from patients who had not received any prior chemotherapy. Fifty-four per cent of the 645 specimens were sputum, and 46 per cent were obtained from the aspiration of gastric contents. Both the sputum and gastric specimens were tabulated in an identical fashion, as outlined in table 1. There appeared to be no significant difference between sputum and gastric specimens in terms of percentage of positive cultures on L-J or RDM-1 medium when they were compared separately in this manner.

* * *

When sputum or gastric specimens (which have been prepared in the manner described above) are simultaneously inoculated into each of two identical culture media, it would be expected that, in the majority of instances, both cultures would yield the same results. Through the action of the law of chance, it would also be expected that on a number of occasions only one of the two implanted cultures would be positive for the microbe sought—in this case, *M. tuberculosis*. However, the distribution of positive and negative reports would be equal between the two cultures. For instance, if each of the 645 specimens in this report was inoculated simultaneously into each of two identical tubes of Löwenstein-Jensen (L-J) medium, and the media were labeled "inoculated first" and "inoculated second," one would expect the number of cultures of tubercle bacilli obtained from the first-inoculated tubes to be similar to the number of cultures of tubercle bacilli obtained from the second-inoculated tubes. In this report, the difference between the number of positive cultures on L-J medium versus the modified oleic-acid-albumin (RDM-1) medium appears to be significant, and encompasses some-

thing more than the law of chance. This is illustrated in the 645 positive specimens in table 1, in which 94.7 per cent were positive on L-J medium and only 54.8 per cent were positive on RDM-1 medium. When only one of two simultaneously inoculated tubes of medium was found to be positive, the L-J medium was positive 88.7 per cent of the time, and the RDM-1 medium was positive only 11.3 per cent of the time. This latter comparison seems significant in that it represents an analysis of 600 cultures from 300 separate sputum and gastric specimens.

After preparing each specimen in the manner described above, 0.5 ml. was inoculated into one tube of L-J medium, and 0.5 ml. was inoculated into each of two Petri dishes of RDM-1 medium. Therefore, twice as much material from the same specimen was used to inoculate the RDM-1 medium. This procedure would tend to increase the chances of obtaining positive cultures on RDM-1 medium. In this respect, the methods used lend further significance to the much greater percentage of positive cultures on L-J medium versus RDM-1 media.

In 402 specimens from patients who had received no previous chemotherapy (*see* table 2), the incidence of cultures positive for tubercle bacilli on L-J medium was 95 per cent. This was 33.3 per cent higher than the incidence of positive cultures on RDM-1 medium in this same group of patients. In 206 specimens from patients who had received chemotherapy for one or more months prior to the implantation of cultures, 39.6 per cent more of cultures positive for tubercle bacilli were recorded on the L-J medium. As noted in table 2, there was no decline in the incidence of positive cultures on L-J medium as the duration of "pre-culture" chemotherapy increased. The incidence of cultures positive for *M. tuberculosis* on L-J medium was just as high after three or more months of chemotherapy. This same situation does not appear to hold true with RDM-1 medium. Approximately 60 per cent of cultures were positive for tubercle bacilli on the RDM-1 medium after zero to one month of chemotherapy, and only 41.4 to 46.2 per cent were positive after three or more months of chemotherapy. It appears that the chance of obtaining a positive culture on RDM-1 medium decreases slightly as the period of chemotherapy prior to inoculation of the culture increases.

In summary: Six hundred and forty-five separate sputum and gastric specimens were simultaneously inoculated into Löwenstein-Jensen (L-J) medium and a modified oleic-acid–albumin (RDM-1) medium. All 645 specimens produced cultures positive for *M. tuberculosis* on one or both of these media. In 300 specimens (46.5 per cent) only one of the two different simultaneously inoculated media was positive.

The ability of the Löwenstein-Jensen medium to support the growth of *M. tuberculosis* was significantly superior to that of the modified oleic-acid–albumin medium described herein. This difference, in terms of percentage of positive cultures, rose further in favor of the Löwenstein-Jensen medium as the duration of chemotherapy prior to inoculation of the cultures was increased.

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September 9, 1959

PRELIMINARY OBSERVATIONS FROM A CONTROLLED TRIAL OF PREDNISOLONE IN THE TREATMENT OF PULMONARY TUBERCULOSIS

Steroids are generally recognized as useful for the treatment of an acutely ill tuberculous patient but are considered to be contraindicated for the patient in whom chemotherapy has been a failure. The role of steroid hormones in the treatment of the wide spectrum of tuberculous patients between these two extremes is being studied by the clinical investigators[1] participating in the U. S. Public Health Service Tuberculosis Therapy Trials. This preliminary report is limited to observations during the first twelve weeks of treatment.

Antimicrobial	Adjuvant			
	Prednisolone (9 weeks)	Prednisolone (5 weeks)	Placebo (9 and 5 weeks)	Total
Streptomycin–pyrazinamide (12 weeks) followed by isoniazid–PAS (20 weeks)	190	184	186	560
Isoniazid–PAS (32 weeks)	193	197	195	585
Total	383	381	381	1,145

Patients selected for the trial by the participating hospitals were randomly assigned to one of the six basic regimens shown in the table above. One-third of the patients received prednisolone[2] for five weeks, and one-third for nine weeks. A placebo, especially prepared to be indistinguishable from prednisolone, was given to the remaining one-third, half of whom received it for five weeks and half for nine weeks. In this report the two placebo groups have been combined, since careful examination revealed no differences in composition or therapeutic results.

Antimicrobial agents were given in the following daily dosages: streptomycin, 1 gm.; pyrazinamide, 35 to 45 mg. per kg. of body weight; isoniazid, 4 to 6 mg. per kg. of body weight; and PAS, 10 to 12 gm. All patients who received PAS initially were given the acid form of the drug for the first twelve weeks to avoid excessive intake of sodium during the period of steroid therapy.

The daily dose of prednisolone was 20 mg. for the first three days, 15 mg. for the next four days, followed by a maintenance dose of 10 mg. During the final week of steroid therapy on all regimens, the dose was gradually reduced to 5 mg. a day for four days and 2.5 mg. for the last three days. Tablets of 2.5 mg. of prednisolone or placebo were given at six-hour intervals.

At the beginning of the trial, supplies of prednisolone and placebo, packaged in individual, sealed, opaque bottles were sent to each hospital. Bottles were identified only by serial numbers, and the contents were known only to the central office. When a patient was selected for the trial, he was assigned the first unused bottle of medication. The seal was then broken and the bottle opened. The duration of the auxiliary medication and whether the patient was to receive streptomycin–pyrazinamide or isoniazid–PAS as initial chemotherapy was written *inside* the bottle cap. With this arrangement neither the patients nor the hospital staff knew whether a patient received prednisolone or placebo, and could not,

[1] Dr. R. C. Edson, Cedarcrest Sanatorium, Newington, Connecticut; Dr. G. C. Wilson, Uncas-on-Thames Sanatorium, Norwich, Connecticut; Drs. R. J. Davies, G. H. Hames, B. L. Brock, and A. G. Lewis, Florida State sanatoriums at Lantana, Orlando, and Tampa, Florida; Dr. R. F. Corpe, Battey State Hospital, Rome, Georgia; Dr. E. G. Beacham, Baltimore City Hospital, Baltimore, Maryland; Dr. H. M. Payne, Middlesex County Sanatorium, Waltham, Massachusetts; Dr. P. T. Chapman, Herman Kiefer Hospital, Detroit, Michigan; Dr. W. J. Steininger, William H. Maybury Sanatorium, Northville, Michigan; Dr. S. Cohen, Glen Lake Sanatorium, Oak Terrace, Minnesota; Dr. C. A. Brasher, Missouri State Sanatorium, Mount Vernon, Missouri; Drs. A. Goldman, B. Friedman, and M. B. Pianetto, Robert Koch Hospital, Koch, Missouri; Dr. C. D. Thomas, North Carolina State Sanatorium, Black Mountain, North Carolina; Dr. H. I. Humphrey, Benjamin Franklin Sanatorium, Columbus, Ohio; Dr. R. H. Browning, Ohio Tuberculosis Hospital, Columbus, Ohio; Dr. H. G. Curtis, Sunny Acres Hospital, Cleveland, Ohio; Dr. G. M. Eckley, Henry R. Landis State Hospital, Philadelphia, Pennsylvania; Dr. George E. Martin, Pittsburgh Tuberculosis Sanatorium, Pittsburgh, Pennsylvania; Dr. Hilarion Sanchez, Sanatorio A. Ruiz Soler, Rio Piedras, Puerto Rico; Drs. P. M. Huggin, E. P. Bowerman, and F. H. Alley, Tennessee Tuberculosis sanatoriums at Knoxville, Memphis, and Oakville, Tennessee; Dr. E. H. Roberts, San Antonio State Tuberculosis Hospital, San Antonio, Texas; and Dr. T. F. Sheehy, Firland Sanatorium, Seattle, Washington.

[2] Generously provided as Delta-Cortef by the Upjohn Company.

without deliberately opening a bottle, know what chemotherapy the next patient would receive.

Clinical, bacteriologic, and roentgenographic examinations of each patient were made before his regimen was assigned and at four-week intervals during treatment. Blood pressure, fasting blood sugar, and symptoms which might be related to drug toxicity were recorded initially and after five, nine, and thirteen weeks of treatment. In the event of intolerance to an assigned drug or severe worsening of a patient's condition which might be checked by a change in chemotherapy, provision was made in the protocol for the responsible physician to change the assigned regimen.

Centrally controlled random assignment of treatment regimens resulted in six similar groups of patients with bacteriologically proved pulmonary tuberculosis, previously treated with antimicrobial agents for no more than two weeks. Two-thirds of the patients are white and there are nearly twice as many males as females. Two-fifths of the patients are less than thirty-five years of age; two-fifths, between thirty-five and fifty-four years of age; and one-fifth, fifty-five years or older.

Eight per cent of the patients had minimal disease; 44 per cent, moderately advanced disease; and 48 per cent, far advanced disease. No cavities were observed in the initial roentgenograms of 29 per cent of the patients, 16 per cent had one cavity each, and 55 per cent had two or more cavities. Tubercle bacilli failed to grow on the initial sputum cultures from 14 per cent of the patients.

Of the 1,145 patients who entered the trial, 114 (10.0 per cent) failed to complete the first twelve weeks of assigned treatment for reasons apparently unrelated to their disease or chemotherapy. Nearly three-fourths of these patients left the hospital against advice. Fortunately,

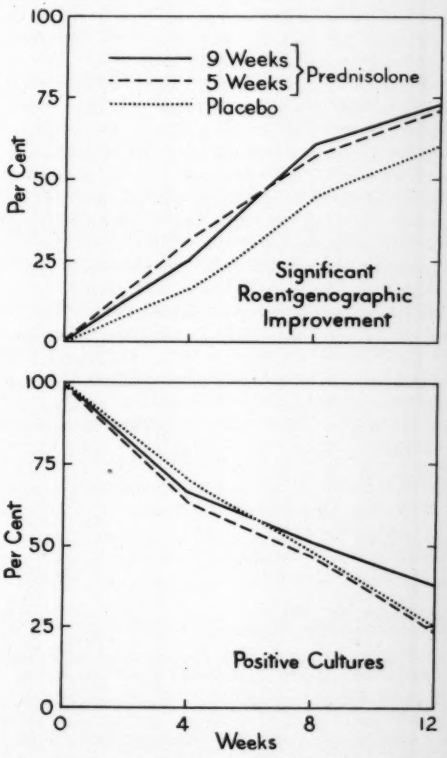

FIG. 1. Roentgenographic and bacteriologic change among patients grouped according to steroid therapy.

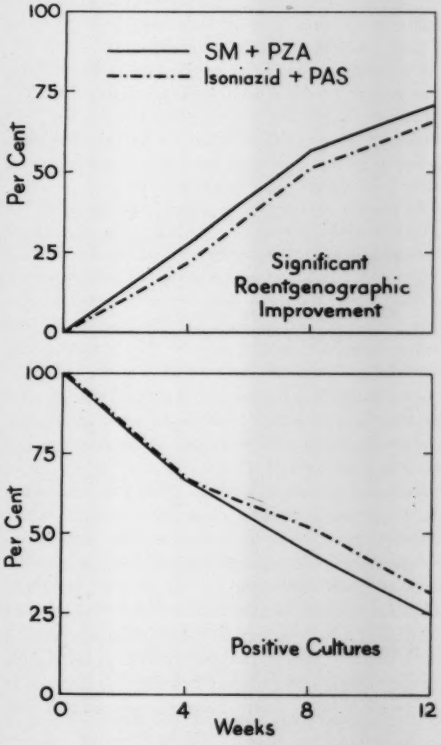

FIG. 2. Roentgenographic and bacteriologic change among patients grouped according to antimicrobial therapy. SM = streptomycin; PZA = pyrazinamide.

these deviations occurred with about the same frequency and involved the same type of patients on each of the regimens.

Toxicity: Among the remaining 1,031 patients, 520 patients received isoniazid–PAS, 2 patients (0.4 per cent) could not tolerate isoniazid, and 30 (5.8 per cent) could not tolerate PAS. Intolerance to PAS occurred with equal frequency in the three regimens in which this drug was used.

Among the 511 patients who received streptomycin–pyrazinamide, 39 (7.6 per cent) could not tolerate daily streptomycin and 18 (3.5 per cent) failed to tolerate pyrazinamide. Intolerance to these drugs occurred somewhat more frequently among patients receiving prednisolone, suggesting that prednisolone, in the dose used, does not prevent intolerance to these antimicrobial drugs.

The adjuvant therapy was discontinued for only 2 patients. Blood sugar levels increased in a seventy-six-year-old male diabetic receiving prednisolone. An eighteen-year-old man developed a rash for which the adjuvant medication was discontinued without knowledge that it was, in fact, placebo.

Therapeutic effect: In figure 1, the patients have been grouped according to their adjuvant therapy: prednisolone for five weeks, prednisolone for nine weeks, or placebo. Approximately half the patients in each group received isoniazid–PAS and the other half streptomycin–pyrazinamide.

As shown in the upper chart, the per cent of patients with substantial (marked or moderate) roentgenographic improvement increased rapidly and steadily in all groups during the twelve weeks of treatment. Patients who received either five or nine weeks of prednisolone fared equally well and had a definite superiority over the patients who received placebo. At the twelfth week, slightly more than 70 per cent of the patients on the prednisolone regimens showed substantial improvement compared with only 60 per cent of the placebo patients.

Reversal of infectiousness, shown in the lower chart, occurred rapidly among all three adjuvant therapy groups. In contrast to the results on roentgenographic change, the bacteriologic results give no indication of any benefit from the addition of prednisolone but instead suggest that continued administration of prednisolone may be undesirable. Among patients given prednisolone for nine weeks, 38 per cent were still infectious in the twelfth week compared with only 24 per cent with the five-week course of prednisolone and 25 per cent with the placebo.

Consideration of both roentgenographic and bacteriologic change suggests that a five-week course of prednisolone is preferable to either the placebo or a nine-week course of the steroid.

In figure 2, the patients have been grouped according to their antimicrobial therapy, either streptomycin–pyrazinamide for twelve weeks followed by isoniazid–PAS for twenty weeks or isoniazid–PAS for thirty-two weeks.

Throughout the twelve weeks of observation, the regimen of daily streptomycin–pyrazinamide produced significant (marked or moderate) roentgenographic improvement in slightly more patients than the regimen of isoniazid–PAS. At the twelfth week, 71 per cent of the patients who received streptomycin–pyrazinamide and 65 per cent of those who received isoniazid–PAS showed significant roentgenographic improvement.

Comparison of bacteriologic change on the antimicrobial combinations, shown in the lower chart of figure 2, shows a more rapid reversal of infectiousness among patients who received streptomycin–pyrazinamide.

These results on both roentgenographic and bacteriologic change confirm the findings of an earlier report[3] that streptomycin–pyrazinamide is the more effective regimen for initial treatment.

In summary: In preliminary observations after twelve weeks of treatment in a controlled trial of prednisolone as an adjuvant to antimicrobial therapy of pulmonary tuberculosis, a five-week course of the steroid was superior roentgenographically and equal bacteriologically to a placebo. On the other hand, a nine-week course of steroid, while also superior roentgenographically to the placebo, resulted in less rapid reversal of infectiousness. Results also confirm earlier findings on the superiority of daily streptomycin–pyrazinamide over isoniazid–PAS as initial therapy.

United States Public Health Service
Tuberculosis Therapy Trials
Tuberculosis Program
Public Health Service
Washington, D. C.

December 30, 1959

[3] United States Public Health Service Tuberculosis Therapy Trials: Am. Rev. Resp. Dis., 1959, *80*, 627.

Correspondence

A PROPOSED METHOD OF TUBERCULOSIS CONTROL

To the Editor of the American Review of Respiratory Diseases:

A recent survey in the city of Dallas indicates that first-grade children from the worst economic and social areas of this city now react to tuberculin only at the rate of 3 per 100. In better areas of the city, the tuberculin reactor rate is as low as 1 in 200. These figures represent a marked change in frequency of infection at the age of six as compared with statistics gathered eight years ago. The figures, however, are not unlike those obtained by testing agencies in other cities and probably are fairly representative of the present rate of infection of children.

It should be pointed out that the assumption can very well be made that most such infections have taken place in the home. Further, the Dallas statistics indicate that, subsequent to the time when a child begins to go to school, he converts his Mantoux from negative to positive at the rate of about 3 per 1,000 per year or, to use the same scale of figures as before, at the rate of about 1 in 300 per year. Such figures indicate a slow, but steady, tuberculinization of the young population at a time when it is beginning to form many other contacts than those of the family itself.

Heretofore, efforts at tuberculosis control have centered upon the discovery of the individual "open case" and his isolation, treatment, and rehabilitation. There can be no doubt that such efforts have been successful in materially reducing the amount of tuberculinization of the youthful population. More recently, on account of the very low yield of new cases obtained in mass roentgenographic surveys, there has been a tendency to adopt surveys of specific groups in the hope of increasing the rate of discovery of new cases. Even in these more pointed programs the method has been less productive than anticipated, owing in part perhaps to the low rate of compliance. The specific groups in whom the yield of newly discovered cases has been highest—the very old, the homeless, and the imprisoned—are not those with whom young children are most likely to come in contact.

Mass skin-test surveys of adult populations followed by roentgenographic examination of positive reactors should result in much higher yields of active cases, but this method calls for three different procedures: injection of material, reading of tests, and roentgenographic follow-up. Compliance rates under such conditions are understandably low.

It is suggested, therefore, that the direction of tuberculosis control be modified in such a way as to make every possible effort to prevent infection of those children who have escaped infection at home. In this way, although the result may be a little slower, it should be possible within a few decades to produce a nontuberculinized population.

The direction of such efforts should be toward extensive surveillance of such people as school teachers, baby sitters, scout masters, college teachers, barbers and beauticians, bus and taxi drivers, and all other persons whose profession or occupation is such as to bring them in contact with considerable numbers of young children.

This kind of modification of a tuberculosis control program has the advantage of being a prophylactic move to preserve the child against even a primary infection. The numbers of persons involved will be comparatively small, so that intensive surveying will be possible. Since many of those involved have to have some type of license, a method of control will be easily established and the expense will not be excessive.

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November 11, 1959

PYRAZINAMIDE–ISONIAZID—TREATED TUBERCULOUS PATIENTS

To the Editor of the American Review of Respiratory Diseases:

In 1954, Campagna and associates[1] reported a series of 21 patients whose tuberculosis improved remarkably while taking pyrazinamide and isoniazid although earlier therapy with streptomycin, isoniazid, and para-aminosalicylic acid had been attended by persistence of tubercle bacilli in the sputum and progression of the disease. Because this series presented some unusual features and because 18 of the original group are still being observed regularly (one is known to have died a cardiac death, while two others have been lost to the study), a further note seems warranted.

Since conclusion of the one-year period of therapy in 1953, none of these remaining 18 patients has had any form of treatment directed against the tuberculosis. Eleven of them are gain-

[1] CAMPAGNA, M., *et al.*: Am. Rev. Tuberc., 1954, *69*, 334.

fully employed while 2 others perform their customary household chores; one is mentally incompetent to care for herself, one has sputum consistently positive for tubercle bacilli despite lack of roentgenographic or other evidence of active tuberculosis, and 3 are disabled by nontuberculous states (emphysema, psychosis, cerebral hemorrhage).

This series is small but provokes questions which must be answered daily for an increasing number of persons whose life span has been extended by specific antimicrobial therapy. The criteria that Myers[2] presented years ago are helpful in this regard:

(a) Should these people be classified as having inactive tuberculosis? Most of our patients capable of full-time work, excreting no detected tubercle bacilli and having no roentgenographic evidences of progressive disease, still exhibit roentgenographically large air-containing structures at the site of unquestioned pretreatment cavities. Are these structures "cysts," "bullae," "healed cavities," or smoldering lesions? One patient has recurring febrile episodes with appearance of fluid levels in a thin-walled structure, but no acid-fast bacilli can be isolated from the sputum even when this increases in amount. Is this a phenomenon of secondary bacterial infection? Should such people have public health supervision and consequent restriction of personal freedom until the "cavity" disappears from roentgenographic view? Must *Diagnostic Standards* be modified to provide for such cases?

Interestingly enough, there is no consistent correlation between the extent of pulmonary destruction as suggested by roentgenographic appearance and degree of ventilatory impairment as measured by pulmonary function testing, nor did any patient seem to have cor pulmonale even with a maximal breathing capacity of less than 40 per cent predicted normal value, a level at which right ventricular hypertrophy has repeatedly been shown to occur in nontuberculous subjects.

(b) Have we kept them from spreading tuberculosis? Only one person is known to excrete tubercle bacilli in the sputum. All of these patients were regarded as "poor hospital material," and the usual fears of permitting them to be outpatients were expressed. Yet, of the relatively few children in contact with them, no one was found to have become tuberculin positive since inception of the project in 1952. This tends to support the sense of security engendered by the failure to isolate tubercle bacilli from the sputum in routine examinations.

(c) Are they socially and economically restored? A frequent indication for extirpative surgery is the belief that permitting a patient to retain a partially or completely destroyed lung jeopardizes his ultimate rehabilitation. No great support for this thesis comes from this study. Eleven of our patients are at full-time work while 2 others perform household duties. Neither the chest film nor the data obtained from testing ventilatory function aided us in predicting whether a given person would be able to work usefully: Of the 9 patients whose cavities had not closed, 4 are at full-time work and 2 at part-time work; of the 9 with presumed cavity closure, 7 work a full day. While *average* values indicate less restriction of breathing capacity with cavity closure, the individual factor still prevails. One man with no demonstrable cavity and a maximal breathing capacity approximately 44 per cent of the computed normal is unable to work because slight effort causes respiratory distress; another with unequivocal roentgenographic evidence of patent cavity and maximal breathing capacity 25 per cent of the predicted normal value is engaged at an eight-hour day of manual labor. Perhaps we ought not to be astonished that the combination of economic necessity and positive motivation is a potent factor for rehabilitation. Unfortunately, we were unable to offer formal rehabilitation procedure to any in this group.

In summary: Of 21 ambulatory patients followed five years after concluding a year of therapy with pyrazinamide and isoniazid,[3] only one has died, presumably of cardiac disease. Eighteen patients are still observed regularly, and 13 of them are at full occupational status. Only one is "sputum positive." There is no evidence that pyrazinamide and isoniazid cause permanent damage in those who escape the early ill effects or who recover from early toxicity on drug withdrawal. There is evidence that this combination of drugs can produce lasting good results when used in chronic pulmonary tuberculosis.

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August 29, 1959

VACCINATION AGAINST TUBERCULOSIS WITH NONLIVING VACCINES

To the Editor of the American Review of Respiratory Diseases:

A symposium devoted to the topic of vaccination against tuberculosis with nonliving vaccines was held in Florence, September 23–25, 1959. The

[2] MYERS, J. A.: J. Thoracic Surg., 1937, *6*, 513.

[3] With no therapy thereafter.

purpose of the meeting was to provide an occasion for workers in this area of investigation to discuss informally the problems and prospects of immunization against tuberculosis with killed preparations.

The Italian participants in the scientific program were Professors G. Buonomini, A. Degli Esposti, G. Mazzetti, G. Petragnani, G. Salvioli, G. Santopadre, and L. F. Signorini. American contributors were Drs. A. J. Crowle, J. Schulmann, D. W. Smith, D. W. Weiss, A. S. Youmans, and G. P. Youmans. Attending from other countries were Drs. S. V. Boyden of the Statens Seruminstitut, Copenhagen; D. Gericke, Farbwerke Hoechst A.G., Frankfurt a. M.; C. N. Iland, Ayrshire Area Laboratory, Scotland; A. Jespersen and M. Magnusson, Statens Seruminstitut; B. Swedberg, University of Göteborg, Mariestad; C. O. Siebenmann, University of Toronto; and G. Vahlne and P. Wahlen, University of Göteborg.

The scientific communications were presented at four sessions concerned, respectively, with the topics of the immunogenicity of killed tubercle bacilli and bacillary extracts; methods of preparation and administration of nonliving vaccines; experiences in human immunization with the Italian preparations Anatubercolina Integrale Petragnani (A.I.P.) and Vaccino Diffondente Salvioli (V.D.S.); and problems of the control and evaluation of nonliving antituberculosis vaccines. The papers were read in Italian or English, the Centro Per La Vaccinazione Antitubercolare of the University of Florence providing for a simultaneous translation service.

It became clear during the course of the meeting that essentially similar findings have been made in the several laboratories represented, despite the variations in the vaccines employed and in the conditions of testing. The conclusions to which these common experiences appear to lead were summarized in the resolution adopted at the close of the meeting:

"Participants of the first international symposium on vaccination against tuberculosis with nonliving vaccines are agreed that a significant degree of enhanced resistance against tuberculosis can be elicited by appropriate vaccines composed of killed tubercle bacilli or certain of their fractions or extracts. It has been common experience that much importance attaches to the choice of the methods of killing and preparation, the dose, route, and vehicle of administration, and the length of the vaccination–challenge interval, in attaining a correct and reproducible evaluation of the protective capacity of nonliving vaccines. Under favorable conditions of administration, certain nonliving preparations are at least as efficacious as vaccines composed of living, attenuated tubercle bacilli. It is also recognized that tuberculin skin hypersensitivity cannot always be considered to be either a condition or a criterion of artificially produced enhanced resistance.

It is the opinion of the participants that antituberculosis vaccination will prove to be more effective than therapy alone in the long-range control of tuberculosis, especially in areas where its incidence is likely to remain high for some time to come."

The scientific communications and discussions also made clear a point long recognized by some investigators: Although relatively minor changes in the procedures of preparation or administration of *some* nonliving vaccines reduce or destroy their immunogenicity, a surprisingly large variety of vaccines derived from killed tubercle bacilli have proved to be immunogenically active under a wide range of test circumstances. These observations encourage, on the one hand, the view that immunogenicity resides in a stable property of the tubercle bacillus and is not an erratic and, for practical purposes, a rather artificial and unreliable phenomenon; on the other hand, they point to the difficulty of choosing from the many active preparations reported the most efficacious, safe, and otherwise desirable vaccine. There was a general concurrence of opinion, therefore, that the eventual development of such a vaccine would be greatly facilitated by the establishment of a central testing laboratory, in which different preparations could be compared under identical conditions.

A final area of unanimous accord was the unique charm of the city of Florence, and the hospitality of the Centro in supporting the costs of the conference and the stay of the participants.

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University of Florence
Florence, Italy

DAVID W. WEISS
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November 30, 1959

Books

TEXTBOOK OF MICROBIOLOGY. By William Burrows, with the collaboration of Richard Janvier Porter, and James William Moulder. Pp. 954, W. B. Saunders Company, Philadelphia, 1959. Price, $14.00.

Burrows' *Microbiology* has a long and excellent history, and its seventeenth edition unquestionably maintains the high standards of its predecessors. The book is generally well written, especially the chapter on bacterial metabolism, and contains numerous illustrative pictures, notably of viruses. The selection of references aids further reading in depth and speaks for the authors' remarkable knowledge of the literature. In a book of such scope, written almost single-handed, one can inevitably find certain omissions and shortcomings, such as the lack of any mention of the unclassified acid-fast mycobacteria in the chapter entitled Mycobacterium, to mention only one. The text is almost all-inclusive, at least in those areas of microbiology of interest and import to the medically oriented reader. Thus, a part of the book is devoted to general aspects of microbiology, such as techniques, morphology and physiology of microorganisms, factors of pathogenicity, and the elements of the immune response and infection immunity. The second part deals exclusively with microorganisms as agents of disease, including bacteria, spirochetes, fungi, rickettsiae, viruses, and protozoan and metazoan parasites. The book is presumably written for both the beginner and the more prepared reader. However, the detailed descriptions of certain techniques, especially those on serology, do not add much to the value of the book; they are too short for the beginner and almost useless for the experienced worker.

Microbiology is a biologic science as well as an applied field of human or veterinary medicine. In consequence, its scope ranges from problems of molecular biology including biochemistry and pathology to those of human behavior and socio-economics as they influence infection. Inevitably, it becomes difficult, if not impossible, to combine all of these in one text of 950 pages and to do justice to the relative importance of the single parts. One has either to become highly selective to preserve depth in some fields, or one has to sacrifice thoroughness for completeness. The latter is still considered in many circles as the correct approach in the teaching of future physicians, whereas it has been discarded long ago in programs for graduate students. Similar difficulties have arisen in other fields, notably biochemistry, and the solution to the problem has been to separate the basic aspects of the discipline from the applied ones. Here one finds texts dealing almost exclusively with basic biochemistry, and clinical chemistry and a systematic treatment of disorders of metabolism are left to the clinical treatise. This does not, of course, preclude the discussion of medical problems as examples for theoretical considerations. It seems that this might also apply to microbiology in which a separation between "general microbiology," including the host response to infection, and "microorganisms as agents of disease" is possible without much difficulty.

Despite these remarks, Burrows' *Microbiology* is a commendable textbook and it will be used widely, especially in the instruction of medical students.

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PREVENTIVE MEDICINE IN WORLD WAR II, MEDICAL DEPARTMENT, UNITED STATES ARMY. Volume IV: Communicable Diseases Transmitted Chiefly through Respiratory and Alimentary Tracts. John Boyd Coates, Jr., Editor in Chief, U. S. Government Printing Office, Washington 25, D. C., 1958. Price, $5.50.

This volume is designated the fourth in a series to comprise the official history of the Medical Department of the United States Army in World War II and deals with those diseases of military importance which are transmitted chiefly through the respiratory and alimentary tracts. Concerning the former category are chapters on chicken pox, common respiratory diseases, influenza, measles, mumps, psittacosis, rubella, small pox, diphtheria, meningococcal meningitis, the pneumonias, hemolytic streptococcal infections, tuberculosis, whooping cough, and coccidioidomycosis. Each is written by an author conversant with his subject matter.

The introduction brings to light a number of facts of interest. Although deaths from disease constituted only 5.1 per cent of total deaths in the Army and for the first time were less than those due to injury sustained either in or out of combat, disease was responsible for 85.2 per cent of ad-

missions to army medical installations, and communicable disease accounted for more than half such illnesses. Respiratory diseases comprised nearly one-third of disease of an infectious or parasitic nature. The importance of these disorders is clearly evident.

In the ensuing chapters, the utility of studying disease in military populations comes to light. The availability of medical records concerning large numbers of humans whose activities are controlled to some extent provides an opportunity for epidemiologic investigation seldom equaled in civilian life and, in a number of instances, advantage was taken of this situation. The changing picture of susceptibility to some respiratory disorders is also evident in the differences manifested in their behavior in World Wars I and II. Increasing contact between rural and urban populations as a result of improved transportation may account in part, at least, for the seemingly smaller number of recruits susceptible to the common exanthems of childhood. In contrast to this is susceptibility to diphtheria in the young adult population.

Although progress was made in the understanding and potential control of influenza, similar advances in the management of other diseases of viral origin were at times less striking because of technical laboratory problems which only recently have progressed toward solution. Significant strides in the control of meningococcal epidemics were achieved through the prophylactic administration of sulfonamides, but the application of the same method failed to control beta hemolytic streptococcal infection because of the emergence of streptococcal mutants resistant to these agents. Control of tuberculosis was facilitated greatly when routine use of roentgenography was introduced in screening new troops and prior to the advent of specific antituberculous therapy. The chapter on coccidioidomycosis is one of the most interesting, describing how much was learned about several aspects of this disease.

Because the biologic data contained in this volume are interspersed among a significant number of statistical facts and of specialized historical data, it will probably be of greater interest to students of epidemiology and of medical military history than to those whose major concern is clinical medicine. For the latter, bibliographic footnotes may provide a source of useful references.

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A Practice of Thoracic Surgery. By A. L. d'Abreu. Pp. 619, The Williams & Wilkins Co., Baltimore, 1958. Price, $19.00.

This important book on thoracic surgery by the well known surgeon of Birmingham is beautifully printed and handsomely illustrated. It is a personal account of Mr. d'Abreu's practices and preferences, with a reasonable amount of space devoted to the views of others. The style of the text is informal and easily readable, if at times discursive. The book will appeal particularly to men in training in thoracic surgery because of the detailed descriptions of almost all of the operations performed in thoracic surgery. Mr. d'Abreu quite frankly appreciates the difficulties inherent in writing a text book of thoracic surgery when the field is moving so rapidly, and avoids the trap of didactic statements. It is fair comment on the expansion of thoracic surgery that, while one-fifth of the volume is devoted to cardiovascular surgery, this allows only the briefest discussion of open-heart procedures. The discussions of pulmonary suppurative disease are good, and the author speaks with great authority of pulmonary tuberculosis, although he shares, with those of us who saw lives saved by pneumothorax and by thoracoplasty in great numbers and with reasonable risks, a certain wonder that they have so completely been displaced.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

The Award of the Trudeau Medal for 1959

The Trudeau Medal for 1959 was awarded to Dr. Edith M. Lincoln at the annual meeting of the National Tuberculosis Association, held in Chicago, Illinois, May 24–29, 1959.
The presentation was made by Dr. W. L. Cooke

DR. COOKE: As you all know, the Trudeau Medal was established in 1926 as an annual award for the "most meritorious contribution on the cause, prevention or treatment of tuberculosis." The Trudeau Medal Committee for this year was composed of two recent Trudeau medalists, Dr. David Smith and Dr. Corwin Hinshaw; a representative from the American Trudeau Society, Dr. William B. Tucker; the chairman of the Ross Medal Committee, Mr. Peter W. Janss; and a private practitioner from West Virginia.

The other members of the committee, being wiser men, have left the honor of presenting the medal to me. The reason I say "wiser men" is because our medalist this year is of the fairer sex and it is awkward to speak of the long time so devotedly served by her. I can only take refuge in Elizabeth Browning's remark: "A woman's always younger than a man at equal years."

Dr. Edith M. Lincoln graduated at Johns Hopkins in 1916 and, after training at Harriet Lane Hospital, moved to New York. Now, as I understand it, this move occurred because her husband, Dr. Asa L. Lincoln, had accepted a residency in New York. When I hear Dr. Lincoln say, "Liggett," in speaking of Dr. A. L. Lincoln, I am sure that now, as at that time, Dr. Edith Lincoln considers her role as wife, paramount. While waiting for her appointment in New York she spent several months helping her husband's father, a physician at Lacy Springs, Virginia, fight the influenza epidemic.

After training at Willard Parker and Bellevue she realized the complete inadequacy of clinics for the diagnosis, treatment, and follow-up of tuberculosis in children. "Girls have curious minds and fain would know the end of everything."[1] Wise were the men at Bellevue who entrusted Dr. Lincoln with the development of an absolutely first-class clinical service for children with tuberculosis in an effort to know the end of everything.

In October, 1930, a study of the pathogenesis of chronic pulmonary tuberculosis in children, based on 1,000 consecutive cases of primary tuberculosis on the Pediatric Service of Bellevue Hospital, was begun. This twenty-five year follow-up was 90 per cent complete by 1947 and afforded the last opportunity, because of the advent of chemotherapy, to study the natural course of the disease in children. Dr. Lincoln's studies were aided by the National Institutes of Health and, beginning in 1941, by the National Tuberculosis Association.

In the late 1930's, studies on the chemotherapy of tuberculosis were begun by Dr. Lincoln, using Promizole®, a synthetic sulfone. Dr. Lincoln and her associates added streptomycin to her studies shortly after it was introduced and they were soon engaged in trials of Promizole and streptomycin independently and in combination in the treatment of acute forms of tuberculosis in children. These investigations were among the most influential of the well-known studies that established the value of these drugs in the therapy of miliary tuberculosis and tuberculous meningitis in children. In 1952, after the introduction of isoniazid, her program was enlarged to include a series of studies with this drug. By that time an element of confidence prevailed in the treatment of acute childhood tuberculosis. Dr. Lincoln joined others in the statement that "tuberculous meningitis is a curable disease." She has published regimens for the effective use of streptomycin and isoniazid in tuberculosis in children, and called attention to the value of isoniazid in preventing the development of tuberculous meningitis in children with primary tuberculous infection. Her papers form part of a large picture on the chemotherapy of tuberculosis that has been developed since 1947, and are looked upon as among the most stimulating in the treatment of this disease in children.

During all of this period of prodigious clinical research, Dr. Lincoln's status as a teacher was more and more recognized. As one former pupil writes: "It is ungallant of me to recall that, as a senior medical student twenty-five years ago, I was enormously impressed by Edith Lincoln as a teacher and we were a pretty tough bunch to satisfy." So today, former associates of Dr. Lincoln have been selected to test the procedures developed by her, in other parts of the world

[1] Elizabeth Browning.

where cases of acute tuberculosis, and particularly tuberculous meningitis, are more common than in the continental United States.

And now, Dr. Lincoln, after a most awkward and inadequate attempt at outlining your achievements in the fields of clinical research and pedagogy, I want to tell you what your work has meant to the private practitioner away from medical centers and in West Virginia just over the hills from Lacy Springs. You have interested an entire medical specialty in a disease which in 1916 usually meant rapid death or was considered to be of no great concern. Our pediatric friends have regained faith and interest in tuberculin testing and this you have encouraged even though it might be through a "patch test" to keep from having to lasso the much-pricked young patient. You have shown the value of long-term follow-up studies in chronic disease and what to expect in tuberculosis over the years. And above all you have prepared the way for thorough evaluation of chemotherapy in children with tuberculosis. Woman has much perseverance and, as I present to you the Trudeau Medal for 1959, I might do well to quote a quatrain by the author of Ben Bolt:

> Less good from genius we may find
> Than that from perseverance flowing;
> So have good grist at hand to grind
> And keep the mill a-going.[2]

May your genius and perseverance combine to answer many more of the riddles concerning tuberculosis in children!

DR. LINCOLN: I imagine everyone who has received the Trudeau Medal has felt as I do that there are so many other people who are more deserving of this great honor.

It is a wonderful thing to receive a medal for doing just exactly what you wanted most to do. During the thirty-four years that I was in charge of the Children's Chest Clinic at Bellevue, our small group of clinicians and investigators really had a good time, and we looked forward to rounds and conferences with eager anticipation. I am deeply indebted to these faithful co-workers, including the long succession of Fellows made possible through the generosity of the American Trudeau Society. Most of these men and women have retained their interest in chest diseases in children, and many are in charge of chest clinics in pediatric centers.

After almost twenty-five years of study and observation, our dreams were fulfilled by the development of successful antimicrobial agents. Only those of us who have lived through the years when a diagnosis of meningitis meant a death sentence, when 20 per cent of the children who entered the tuberculosis ward at Bellevue died within a year, can realize the impact of the discovery of streptomycin. The end of an era came when the death rate from tuberculosis on our ward dropped below 1 per cent.

When antimicrobial drugs first became available it was obvious that a proper study of their action in children would mean devoting full time to the Chest Service. My husband gave me the encouragement and backing which permitted me to take the job as a volunteer.

The pediatrician has been in the forefront of the fight against tuberculosis. Von Pirquet devised the first practical tuberculin test. Edwards Park, Bela Schick, and Charles Hendee Smith, by their teaching and writing, helped to counteract the belief that tuberculous infection in children was benign. Arvid Wallgren, Professor of Pediatrics in Stockholm, has had a profound influence on everyone interested in the diagnosis and prevention of tuberculosis in children. Armand Delille, French pediatrician, was the first to stress the importance of gastric lavage in recovering tubercle bacilli.

To me this award is a recognition of the contributions of pediatricians in the fight against tuberculosis.

[2] Thomas Dunn English.

Additions to Organization and Committee Structure

The following committees of the American Trudeau Society were appointed in November, 1959:

Committee on Smoking and Health

Robert H. Browning, M.D., *Chairman*
Ohio Tuberculosis Hospital
Columbus 10, Ohio

Martin M. Cummings, M.D.
Department of Microbiology
University of Oklahoma School of Medicine
800 Northeast 13 Street
Oklahoma City 4, Oklahoma

Philip E. Sartwell, M.D.
615 North Wolfe Street
Baltimore 5, Maryland

Committee on Diagnostic Standards in Nontuberculous Respiratory Diseases

H. William Harris, M.D., *Chairman*
4599 Highland Drive
Salt Lake City, Utah

Attilio D. Renzetti, Jr., M.D.
Veterans Administration Hospital
Syracuse 10, New York

George R. Meneely, M.D.
Vanderbilt University Hospital
Nashville 5, Tennessee

John D. Steele, M.D.
Veterans Administration Hospital
San Fernando, California

John P. Wyatt, M.D.
1402 S. Grand Avenue
St. Louis 4, Missouri

Committee on Appraisal of Programs

Roger S. Mitchell, M.D., *Moderator*
4200 East Ninth Avenue
Denver 20, Colorado

William L. Cooke, M.D.
230 Professional Building
Charleston, West Virginia

William R. Barclay, M.D.
950 East 59 Street
Chicago 37, Illinois

William B. Tucker, M.D.
Veterans Administration
Washington 25, D. C.

John S. Chapman, M.D.
5323 Harry Hines Boulevard
Dallas 35, Texas

Frederick C. Warring, Jr., M.D.
Laurel Heights Hospital
Shelton, Connecticut

Advisors

John B. Barnwell, M.D.
Veterans Administration
Washington 25, D. C.

David T. Smith, M.D.
Duke Hospital
Durham, North Carolina

Subcommittee on Unclassified (Atypical) Acid-fast Bacilli
(of Committee on Therapy)

William Lester, M.D., Chairman
5515 County Line Road
Hinsdale, Illinois

Ernest H. Runyon, Ph.D.
Veterans Administration Hospital
Salt Lake City 13, Utah

Lydia Edwards, M.D.
Division of Special Health Services
Public Health Service
Washington 25, D. C.

Obituary

Julian A. Moore

1896–1959

Dr. Julian A. Moore, a pioneer in thoracic surgery, died unexpectedly of a heart attack in his office at Asheville, North Carolina, on November 7, 1959. Dr. Moore was born in Abbeville, Georgia, on October 31, 1896. He received his early and undergraduate education at Wilmington, North Carolina, and at the University of North Carolina, from which institution he was granted the degree of Bachelor of Science in Medicine in 1916. His medical degree was obtained from the University of Pennsylvania in 1918. Following an internship in the Chestnut Hill Hospital in Philadelphia, Dr. Moore entered the practice of medicine at Wilmington, North Carolina, in 1921 and remained there until 1926. During 1927 and 1928 he was a student in the Postgraduate School of the University of Pennsylvania and was graduated with the degree of Master of Science (Surgery) in 1929. In 1921 he was married to Miss Emogene Bellamy of Knoxville, Iowa, who survives. In addition to Mrs. Moore, he is survived by a son, Robert French Moore, and a daughter, Mrs. Richard Young Moon of Asheville, North Carolina.

From July, 1928, to October, 1929, Dr. Moore with Dr. Frank S. Dolley served as the first residents on the service of Dr. John Alexander at the University Hospital in Ann Arbor, Michigan. Here he acquired the precepts of thoracic surgery which he later used to such good advantage for the people of the Southeastern area. Dr. Moore began his surgical practice in Asheville, North Carolina, in 1930, and remained as a leader in the field of surgery and in community affairs until his death.

His contributions to the development of the surgical treatment of tuberculosis were innumerable. Dr. Moore designed the Moore thoracoscope, which was a standard in the armamentarium of the thoracic surgeon for many years. In addition to his busy surgical practice, Dr. Moore served as Chief of Surgery at the Western North Carolina Sanatorium, where a wing was dedicated in his honor in 1953, and as a consultant at the Veterans Administration Hospital in Oteen, North Carolina. He acted also as a consultant to the State Board of Rehabilitation. One of the last of his numerous scientific papers was presented at the Southern Surgical Association meeting in 1957 and was a record of a ten-year follow-up of 1,080 patients who had 1,130 resections for pulmonary tuberculosis.

During his busy life Dr. Moore had many honors bestowed upon him. He was a member of Phi Alpha Sigma Medical Fraternity and of Alpha Omega Alpha, honorary scholastic medical fraternity. In 1946 Dr. Moore was elected President of the Buncombe County Medical Society, and in 1953 he was honored with the Presidency of the Tenth District Medical Society. He also served as Vice-President of the Medical Society of North Carolina. Among other honors, Dr. Moore received citations from the President of the United States and a Governor of North Carolina for outstanding contributions to the physically handicapped.

In addition to membership in the State and County medical societies, he was a member of the American Medical Association, the American Association for Thoracic Surgery, the American College of Surgeons, the Southern Surgical Association, the American Trudeau Society, the John Alexander Society of Thoracic Surgery, and the Southeastern Surgical Congress. He was a Diplomate of the American Board of Surgery and one of the founders of the Board of Thoracic Surgery.

Due to Dr. Moore's leadership, the library of the Buncombe County Medical Society was established in 1935. The Medical Society, in cooperation with representative leaders of the community, is now completing plans for the Julian A. Moore Study Room to be established in the library in his memory.

This obituary could not be closed without an expression of the author's sense of deep personal loss in the death of Dr. Moore. Since 1943, when he was at the peak of a busy surgical career and while the demands upon his time and energies were innumerable, he was a true friend and counselor. Without his continuing help and interest it would not have been possible for the Surgical Service at the Oteen Veterans Administration Hospital to have developed to its present standing.

His influence in community affairs, in surgery, and in teaching will be felt for many years to come.

JAMES D. MURPHY

NOTICES

The following dates and locations of the next five annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1960—Los Angeles, California—May 15–20
1961—Cincinnati, Ohio—May 21–26
1962—Miami Beach, Florida—May 20–25
1963—Denver, Colorado—May 12–17
1964—New York, New York—May 24–29

POSTGRADUATE COURSE IN "EFFECTS OF AIR POLLUTION AND ENVIRONMENT ON HEALTH"

A postgraduate course in "Effects of Air Pollution and Environment on Health" will be presented at the Wisconsin Center Building of the University of Wisconsin, May 19–20, 1960. The course Director is Seymour B. Crepea, M.D., Associate Professor of Medicine and Director of the Allergy Clinic at University Hospitals. This will be a clinical discussion on various phases of air pollution and their effect on health. Industrial pollution, air-borne infections, and problems of rural air pollution will be considered. Speakers and discussants will be drawn from the United States Public Health Service, the Veterans Administration, and several medical schools in addition to the University of Wisconsin.

Requests for information and registration should be sent to Dr. Robert C. Parkin, Coordinator of Graduate Medical Education, University of Wisconsin Medical School, 418 N. Randall Avenue, Madison 6, Wisconsin.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE

VOLUME 81 April 1960 ABST. NO. 4

The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 207 journals which represent 31 countries and which are published in 18 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Bronchiolitis After Shot Firing in a Colliery. F. L. KRONENBERGER*. *Brit. J. Dis. Chest*, July, 1959, 53: 308–313. (* Chest Clinics, North Northumberland, England)

The cases are reported of two miners who were exposed to fumes from shot firing. These miners used as an explosive "Unigel," which is a gelignite type of explosive and contains nitro compounds which release nitrous fumes. Both suffered from bronchiolitis and recovered. Pulmonary edema and bronchiolitis caused by the inhalation of nitrous fumes in grain silos have been observed in recent years in the U.S.A., and have been called "silofiller's disease." The roentgenograms of the American farmers and the Northumbrian miners showed the same picture of nodular opacities. Those of the farmers who had steroid therapy responded dramatically, and one miner also responded immediately to prednisolone. The problems of nitrous fume poisoning in industry, particularly in mining, and the relevant literature are discussed.

M. J. SMALL

Some Pilot Observations on Oesophageal Carcinoma Deaths in Mental Patients. F. A. NASH*. *Brit. J. Cancer*, June, 1959, 13: 164–167. (* Western Hosp., Fulham, London, England)

Among 144 deaths from carcinoma at Friern Mental Hospital from 1949 to 1956, 12 or 8.3 per cent were esophageal, as compared to about 2.5 per cent in the general population of England and Wales. This relative excess was not explained by age and sex differences in the 2 groups. The proportion of esophageal cancer deaths was directly related to the length of stay in the hospital: 3.4 per cent in "short-stay" patients (less than 10 years), 16.0 per cent in "long-stay" patients. It appears that there may be an etiologic factor in the very chronic patient or, more likely, in his environment which favors the development of esophageal carcinoma. Suggested possibilities are the long-term consumption of paraldehyde, lubricant for tube-feeding, et cetera.

W. J. STEININGER

Cytologic Examination of Sputum and Bronchial Washings in Carcinoma of the Lung (in Swedish). L.-G. WIMAN*. *Nord. med.*, July 16, 1959, 62: 1081–1084. (* U. of Uppsala, Sweden)

A preliminary report is given of a new cytologic staining technique based on the demonstration of a high concentration of protein-bound sulfhydryl groups in cancer cells. The method, first described by Barrnett and Seligman, later modified by Bahr, has previously been used only on sections. When applied to exfoliative cytology this technique reveals a distinct hyperchromasia in the malignant cells, which are easily detected under the microscope at a low power magnification. The usual criteria of malignancy are also seen. Microphotographs are presented. With the new method, time and work are saved and the final diagnosis will probably be more accurate.

J. HAAPANEN

Involvement of the Lungs in Darier's Disease (in German). U. KRÖGER* and R. W. MÜLLER. *Tuberkulosearzt*, July, 1959, 13: 479–483. (* Waldstr. 2, Bad Godesberg, Germany)

A case is described of keratosis follicularis, or Darier's disease, with involvement of the lungs. This is apparently the fourth case described in the literature. The coarse, partly infiltrating and partly circumscribed lesions did not respond to massive antimycobacterial treatment. Administration of vitamin A was followed by considerable improvement of the condition.

J. HAAPANEN

Subcutaneous, Mediastinal and Probable Subpericardial Emphysema Treated with Recompression. J. C. NORMAN* and P. J. RIZZOLO. *New England J. Med.*, August 6, 1959, 261: 269–272. (* Columbia-Presbyterian Med. Center, N. Y., N. Y.)

A case of subcutaneous, mediastinal, and possible subpericardial emphysema is presented. The latter member of this triad has not been reported previously as a result of dissecting extravasated alveolar air. Etiology, pathogenesis, and treatment involving recompression (the third such case in the American literature) are discussed. The possibility of continued and increased occurrence of similar cases in the civilian population as a result of the current spreading popularity of "Scuba" diving is discussed. It has been estimated that more than 10 million Americans now engage in some form of skin diving. Perforce, the medical problems involved in underwater compression and decompression and the omnipresent dangers of air embolism, pneumothorax, and mediastinal, subcutaneous, and possible subpericardial emphysema, will no longer remain clinical and roentgenographic oddities.

M. J. SMALL

Empyema in Infants and Children as a Clinical Problem. T. M. CAMPBELL, JR.*, M. E. JENKINS, and R. B. SCOTT. *J. Nat. M. A.*, September, 1959, 51: 344–349. (* Howard U. College of Med., Washington, D. C.)

Eight infants and children with empyema were treated on a Pediatric Service over a 2-year period.

Cough and rapid respiration were noted in all 8, fever in 7, leukocytosis and anemia in 5, and cyanosis in 4 patients. Chest roentgenograms revealed evidence of pleural effusion with fairly uniform homogenous opacification in all 8 cases. Pyopneumothorax was observed in 3 patients. Thin-walled, cyst-like cavities (pneumatoceles) are commonly observed in roentgenograms taken after the initial stage. They usually produce no symptoms and persist for weeks or months, gradually decreasing in size.

Medical treatment is chiefly concerned with the use of antimicrobials, and these patients received various drugs, including massive doses (1 to 2 million units) of penicillin daily, chloramphenicol, and erythromycin. The newer antimicrobials (novobiocin, kanamycin, vancomycin, and ristocetin) are believed to be promising. Water-sealed drainage with suction was used in 7 of the 8 cases. Surgical drainage should be considered immediately when empyema complicates a pulmonary infection in the very young infant or small child.

L. HYDE

Syndrome of Erythema Nodosum, Bilateral Hilar Enlargement and Polyarthritis. K. RAJASURIYA*, N. NAGARATNAM, and M. SOMASUNDERAM. *Brit. J. Dis. Chest*, July, 1959, 53: 314–318. (* U. of Ceylon)

The syndrome of erythema nodosum, bilateral hilar adenopathy, and polyarthralgia is not rare. Whether it is a disease *sui generis* or a manifestation of some other disease process, its etiology remains uncertain. The onset is usually acute, with constitutional disturbances such as fever and malaise followed by polyarthralgia, erythema nodosum, leukocytosis, and raised sedimentation rate. Roentgenographic examination of the chest reveals a bilateral hilar enlargement, usually symmetrical and without involvement of the lung fields. There is no previous history of upper respiratory tract infection. The arthralgia and erythema nodosum disappear when the fever subsides. The hilar adenopathy usually lasts about 8 weeks, with a range of 2 to 6 months. The Mantoux reaction is negative or weakly positive.

This paper reports two such cases and concludes that sarcoidosis appears to be the most likely cause of this syndrome although only a long-term follow-up of these patients will provide confirmatory evidence.

M. J. SMALL

Diffuse Progressive Interstitial Fibrosis of the Lungs. K. A. LARSEN*. *Acta path. et microbiol. scandinav.*, No. 2, 1959, 45: 167–185. (* Ullevah Hosp., Oslo, Norway)

In 5 cases of diffuse progressive interstitial fibrosis of the lungs the main symptoms were progressive dyspnea, cough and, later, cyanosis. In some patients there were clubbing of the fingers and watch-glass nails. In the later stages, signs of failure of the right ventricle appeared. Pathologically, the lungs were firm, sometimes rubbery, with microscopic disappearance of respiratory epithelium and compact fibrosis with marked development of reticulin fibrils. Small cystic spaces lined with epithelium similar to that in bronchioles also were formed. It is considered highly probable that the primary process was a viral infection affecting the bronchioles and more distal air spaces followed by relatively extensive epithelial necrosis and also, at times, necrosis of the interstitial mesenchymal tissue in the same areas. Such extensive necrosis may lead to collapse of the reticular framework in the alveolar walls making orderly regeneration of the lung lobules with the respiratory elements impossible.

E. DUNNER

Pulmonary Disability in the Hurler Syndrome (Lipochondrodystrophy). A Study of Two Cases. J. F. MURRAY*. *New England J. Med.*, August 20, 1959, 261: 378–382. (* U. of California School of Med., Los Angeles)

The Hurler syndrome (lipochondrodystrophy, or gargoylism) is a rare disease with striking manifestations in the fully developed case. Typical features include numerous skeletal deformities, deafness, hepatosplenomegaly, and cardiac involvement. Two cases of this syndrome are presented with pulmonary function data on 1 patient and autopsy findings on another for the purpose of emphasizing the respiratory aspects of this disease. Pulmonary insufficiency is an important component of the Hurler syndrome and has not received sufficient attention in the past. In addition, the value of tracheostomy for the relief of airway obstruction and improving alveolar ventilation in the Hurler syndrome is demonstrated. It is concluded that respiratory disability is one of the most important aspects of this syndrome.

M. J. SMALL

Echinococcosis of the Diaphragm (in Russian). D. A. BARKOV*. *Khirurgija*, August, 1959, 8: 89–93. (* Kuibishev Med. Institute, U.S.S.R.)

Invasion of muscles by Echinococcus is rare. In a series of 375 cases reported by Petuchov, muscle tissue was involved in 2.6 per cent. The author reports a case of involvement of the left hemithorax characterized roentgenographically by a semicircular shadow over the left diaphragm. The diaphragm was exposed transthoracically. The dome of the diaphragm was covered with cysts containing daughter cysts. Anteroposteriorly the cyst measured 14–16 cm. and extended into the mediastinum, behind the central tendon

and into the right diaphragm. The cyst was resected and the defect in the diaphragm closed by suturing. Recovery was uneventful.

W. A. ZAVOD

On the Diagnosis of Sarcoidosis Illustrated by a Case with Pulmonary and Cardiac Localization. P. B. GRAVESEN*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 127–131. (* Frederiksborg AMTS Centralsygehus, Hillerod, Denmark)

The case is presented of a 20-year-old schoolteacher with pulmonary sarcoidosis who 16 years later developed myocardial sarcoidosis with heart block. The frequency of myocardial granulomas in patients with sarcoidosis emphasizes the importance of electrocardiography in this condition.

W. J. STEININGER

Acquired Hemolytic Anemia Secondary to Boeck's Sarcoid: Report of a Case and Review of the Literature. W. O. WEST*. *New England J. Med.*, October 1, 1959, 261: 688–690. (* Ohio State U. College of Med., Columbus)

The second case of acquired hemolytic anemia secondary to Boeck's sarcoid with involvement of the spleen is reported, and the available literature reviewed. The diagnosis of sarcoidosis was not initially apparent and was made by mediastinal lymph node biopsy. The only findings were hemolytic anemia, mediastinal lymphadenopathy, and splenomegaly. Remission of hemolysis occurred after splenectomy. The need to search for a primary process in every case of acquired hemolytic anemia is emphasized. The diagnosis of hemolytic anemia and the pathophysiology of hypersplenic syndromes are discussed.

M. J. SMALL

The Clinical Application of Paper Electrophoresis in Sarcoidosis. N. LEVITT*. *Dis. Chest*, September, 1959, 36: 243–247. (*Harper Hosp., Detroit, Michigan)

The serum electrophoretic patterns of 25 patients with sarcoidosis (proved by tissue biopsy) were studied. The electrophonetograms were characterized by an increase in the alpha 2-, beta-, and a marked increase in the gamma globulin, while the albumin showed a decrease. These differed markedly from the patterns observed in cancer of the lung, leukemia, congestive failure, and active tuberculosis. No conclusions could be drawn regarding the effect of the steroids on the patterns because of too few cases and too short a period of observation.

E. A. ROUFF

Pachypleuritis with Formation of Calcified Plaque and Bronchial Fistula. A. TUXEN*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 232–240. (* Vardasen Sanatorium, Norway)

In the course of later years, patients who have had pleurisy may develop calcified pleural plaques, heretofore generally regarded as innocuous. Twelve patients are reported who, however, 23 to 36 years following their pleurisy, developed bronchial fistula and limited empyema associated with calcified pleural plaques. Recurrence of disease was characterized by acute fever, chest pain, and cough, and tubercle bacilli were found in the sputum in all instances. Bronchiectasis was demonstrated in most cases. Prolonged specific antimicrobial and conservative pre- and postoperative treatment is necessary.

W. J. STEININGER

Interstitial Pneumonia and Its Consequences in Rheumatoid Disease. B. CRUICKSHANK*. *Brit. J. Dis. Chest*, July, 1959, 53: 226–236. (* Glasgow Royal Infirmary, Scotland)

Clinical, roentgenographic, and pathologic features are described in 8 fresh examples of interstitial pneumonia, pulmonary fibrosis, or honeycomb lung associated with rheumatoid disease. The pathologic changes were nonspecific, except in 1 patient who had minute rheumatoid granulomas. The histologic findings are compared with those in previously recorded examples, and it is suggested that the development of interstitial pneumonia and fibrosis leads to bronchiolectasis, which may be of limited or widespread distribution. Honeycomb lung represents the extreme form of the lesion.

The etiology of the lesions is discussed, with particular reference to irradiation, systemic lupus erythematosus, diffuse acrosclerosis, polyarteritis nodosa, and the Hamman-Rich syndrome, and it is concluded that the lung lesions are manifestations of rheumatoid disease (after author's summary).

M. J. SMALL

Artificial Respiration in the Therapy of Primary Varicella Pneumonia. J. D. DIMASE*, R. GROOVER, and J. E. ALLEN. *New England J. Med.*, September 10, 1959, 261: 553–555. (* Cincinnati General Hosp., Ohio)

During the past 10 years, 11 patients with varicella pneumonia were admitted to the Contagious Service of Cincinnati General Hospital. All were acutely and severely ill. Despite the apparent severity at the time of admission, all of the patients recovered with supportive therapy. Adequate oxygenation and humidification seemed to be the most significant factors in treatment. A

twelfth case of primary varicella pneumonia is presented. It was believed that this patient was more critically ill than any of the cases previously seen. The combined use of sedation, the tank respirator, and nasal administration of oxygen was life-saving in this case and should prove a valuable adjunct in the treatment of selected cases of primary varicella pneumonia.

M. J. SMALL

A Study of Pneumothorax Therapy and Its Long-Term Results. I. ALONEN* and J. N. MAKIPAJA. *Acta. tuberc. scandinav.*, No. 3, 1959, 37: 175–186. (* Central Sanatorium of Middle Finland, Kinkomaa)

The long-term results of 401 pneumothorax procedures in 389 patients were analyzed. Of the total series, 68.5 per cent are now capable of work. Of the patients whose pneumothorax was maintained for an adequate time, 83.4 per cent are working. Complications during pneumothorax therapy occurred in 54.3 per cent of the cases, slightly more often when the pneumothorax was incomplete. The most frequent complications were pleural effusion and contraction; empyema occurred in 3 per cent. Almost without exception, impairment of lung function was associated with the presence of pleural effusion and contraction. The result of treatment was unfavorable considerably more frequently in cases with an exudative process. Tuberculosis was the cause of death in 10.5 per cent of the patients with pneumothorax; 90 per cent of these cases were exudative. Pneumothorax can well be retained as a therapeutic procedure since it is a reversible procedure and complications can be fairly readily managed with modern medical therapy.

W. J. STEININGER

Sjøgren's Syndrome: Report of a Fatal Case with Pulmonary and Renal Lesions. U. G. BUCHER* and L. REID. *Brit. J. Dis. Chest*, July, 1959, 53: 237–252. (* Brompton Hosp., London, England)

In 1933, Sjøgren gave a full clinical and pathologic description of a syndrome of which only single features had previously been reported. The triad by which he characterized the condition consisted of keratoconjunctivitis sicca, xerostomia, and recurrent swelling of the parotids; but he stressed the generalized nature of the condition in pointing out that these were often accompanied by rheumatoid arthritis and, less frequently, by minor manifestations in other organs. Although slowly progressive, Sjøgren's syndrome is hardly ever fatal and only 10 autopsies have been reported. The present case is reported not only because the patient came to autopsy but because hitherto undescribed lung lesions were found, together with severe renal damage. The patient, a girl, died at the age of 22 from massive cerebral hemorrhage. The syndrome was diagnosed at 13 when the classical triad developed, together with joint pains and scoliosis.

At autopsy the lacrimal and salivary glands showed the characteristic atrophy of parenchyma with lymphatic infiltration, a similar picture being seen in the mucous glands of the bronchi and esophagus. In the lungs a type of lesion not previously described was found—nodules of hyaline material several centimeters in diameter and associated with arteritis, as well as smaller, probably earlier, changes of exudative pneumonitis with giant cell arteritis. Arterial changes were confined to the lungs and involved the smallest arterioles as well as larger vessels. The kidneys showed varying stages of glomerulitis and interstitial infiltration with plasma cells and lymphocytes.

M. J. SMALL

Hereditary Hemorrhagic Telangiectasia and Pulmonary Arteriovenous Fistula. Survey of a Large Family. C. H. HODGSON*, H. B. BURCHELL, A. GOOD, and O. T. CLAGETT. *New England J. Med.*, September 24, 1959, 261: 625–636. (* Mayo Foundation, Rochester, Minnesota)

Two hundred and thirty-one members of a large family were surveyed for hereditary telangiectasia, and 39.4 per cent were found to have it. A significant number of members of the family suffered from important complications of telangiectasia, such as bleeding and disturbances of the central nervous system. Fourteen of the 231 members of the family were found to have pulmonary arteriovenous fistulas; 6 of the 14 had symptoms from the fistula. All members of families known to have hereditary telangiectasia should have periodic roentgenographic studies of the thorax in a search for fistula whether or not they have symptoms.

The only satisfactory treatment for pulmonary arteriovenous fistula is surgical removal. No more lung tissue should be removed than is necessary because other fistulas may develop subsequently and require removal. All fistulas do not need to be removed, but surgical intervention seems indicated for enlarging lesions, hemoptysis, hemothorax, polycythemia, bloodstream infections, and abscesses. Two persons, each from a family having hereditary telangiectasia, should not marry each other and have children, for a lethal form of the disease is likely to be passed on to the children.

M. J. SMALL

Treatment of Severe Tetanus. J. R. Lawrence* and M. J. W. Sando. *Brit. M. J.*, August 1, 1959, No. 5143: 113–117. (* U. of Adelaide, South Australia)

The treatment of 9 cases of severe tetanus by paralysis with *d*-tubocurarine and artificial respiration is described. Four patients died, 2 of them from pulmonary emboli and another in acute renal failure. Several other complications occurred, including bone marrow depression, thought to be related to prolonged nitrous oxide administration, and hyperglycemia. This technique relieves the distressing muscular hypertonus of tetanus and prevents spasms. It allows adequate pulmonary ventilation and aspiration to be performed, thus avoiding the respiratory complications which commonly result from the rigid thorax and inadequate cough of patients with severe tetanus. Feeding and nursing care are made easier. However, because of its complexity, the method should be reserved for fulminating cases or for those in which sedatives and other relaxant agents do not prevent reflex spasms. It requires careful continuous medical supervision and skilled anesthetic control. Tetanus in the more advanced communities should be treated by special units with adequate facilities, and paralysis with artificial respiration should be available for the severe case (Authors' summary).

E. A. Riley

Tracheostomy in Childhood. A. W. Venables*. *Med. J. Australia*, August 1, 1959, 2: 141–143. (* Royal Childrens' Hosp., Melbourne, Australia)

Tracheostomy may be indicated for the relief of respiratory tract obstruction due to a wide variety of conditions which may be divided into two groups. The first group includes conditions in which there is actual mechanical obstruction for which tracheostomy provides a "by-pass." The second group includes conditions in which obstruction is due to pooling of secretions in the respiratory tract. Other factors, such as unconsciousness, pain, or paralysis, contribute to this.

Mechanical obstruction may occur in a child as a result of acute infective laryngotracheobronchitis, epiglottitis, or diphtheria; foreign body, trauma (including endoscopy), congenital anomalies, tumors, hemorrhage, allergic edema, bilateral recurrent laryngeal nerve lesions, "laryngeal spasm"; and from extrinsic pressure, e.g., congenital goiter. Secretional obstruction may occur with head injury, cerebral tumor, encephalitis, tetanus, poliomyelitis, polyneuritis, and chest injuries.

Indications for tracheostomy include actual or potential obstruction. In the latter event, the indications are concerned with the nature and severity of the patient's primary condition and with its likely effect on respiratory function. Stridor, retraction, impaired ventilation, and anoxia (overt or subclinical) constitute major indications. Severe oxygen lack may occur without cyanosis, and restlessness, disturbed sleep patterns, and fatigue may result from oxygen lack. Sedatives may be definitely harmful in this situation. Deterioration of the patient's condition under observation, and fatigue or potential fatigue in a patient with airway obstruction should be treated with tracheostomy at once, and by the nearest available personnel.

Management of the patient with a tracheostomy must include constant supervision to ensure the continued patency of the airway. Secretions must be aspirated, the air must be humidified, the patient's fluid balance carefully supervised, and the position of the tube must be controlled at regular intervals.

Decannulation must be planned with a view to the condition requiring tracheostomy in the first place. In an acute process, decannulation may usually be performed within a few days, after several successive trials with the tube occluded. Once the tube is removed, the incision may remain open for a while, and close attention must be paid to the patient until normal breathing has resumed. The period immediately after removal of the tube is one of particular danger in infants, even if occlusion was well tolerated. This is especially true if removal of the tube was delayed and the tracheal rings have softened. If the tube has been in place for a long time, it must be remembered that the fibrous tissue tends to contract rapidly and may completely occlude the opening in an hour or two. For this reason, an attempted decannulation after a long period of tracheostomy breathing should be preceded by excision of the fibrous tissue in case replacement of the cannula becomes necessary.

H. Simon

Mesotheliomas of the Pleura. M. W. Wolcott*, W. A. Shaver, H. E. Walkup, and E. D. Peasley. *Dis. Chest*, August, 1959, 36: 119–126. (* V. A. Hosp., Coral Gables, Florida)

Mesotheliomas are controversial tumors. In general, three concepts of origin are in vogue. One expresses the belief that there is no tumor of mesothelial origin and that all so diagnosed are in reality metastatic carcinoma primary in the lung. The second divides these tumors into solitary pleural tumors, both benign and malignant, and a diffuse type which is always malignant. These authors believe the solitary tumors are mesenchymal in origin and arise from subpleural

tissue, while the diffuse tumor is mesothelial in origin arising from the living cells of the pleura. The third group believes that both the solitary and diffuse types are mesothelial in origin. The present discussion deals with 2 solitary and 6 diffuse neoplasms.

E. A. ROUFF

TUBERCULOSIS, PULMONARY

Criteria for Use of the Term "Inactive" in Pulmonary Tuberculosis Treated with Antimicrobials (in Portuguese). L. CARRIJO*, M. LIMA-FILHO, M. F. FARIA, and M. CONDE. *Rev. paulista tisiol.*, May–June, 1959, 20: 201–207. (* Dispensario do Ipiranga, São Paulo, Brasil)

From 202 patients who were treated with antimicrobials and who had sputum repeatedly negative by direct examination, 530 samples of sputum or bronchial lavage were cultured. Only 4 specimens from 4 patients were positive. Thus, most cases with sputum negative by direct examination were not "inactive" according to the criteria of the U.S. National Tuberculosis Association. On the other hand, 1 patient with sputum negative by direct examination, culture, and animal inoculation developed empyema 11 months later. Recognizing the N.T.A. requirements, but considering the cost of culture and animal inoculation, it is proposed to label as inactive those cases with sputum repeatedly negative, adding the proviso "without culture"; whereas, those negative according to the requirements of the N.T.A. should be labeled inactive "with culture."

J. F. CARNEIRO

Bronchial Abnormalities after Primary Tuberculosis. L. E. HILL* and J. E. G. PEARSON. *Brit. J. Dis. Chest*, July, 1959, 53: 278–295. (* Frenchay Hosp., Bristol, England)

Between 1948 and 1954, 176 children were admitted to hospital for the treatment of primary tuberculosis. Of these, 116 showed evidence of pulmonary collapse and were, therefore, considered suitable for further investigation by bronchography. This investigation was carried out in 78 cases. The records were studied of 562 patients admitted to the thoracic unit in the 10-year period 1946–1955 who were diagnosed as suffering from bronchiectasis.

Bronchiectasis develops in 40.8 per cent of children after primary tuberculosis which gives rise to pulmonary collapse. A further 14 per cent show bronchial irregularity. Stricture formation is rare. At least 2.1 per cent of children with a pulmonary primary tuberculous complex develop bronchiectasis. The younger the child affected with pulmonary collapse and the longer the duration of such collapse, the more likely is bronchiectasis to develop. The right upper and middle lobes are most frequently affected. The majority of children who develop bronchiectasis after primary tuberculosis are symptom-free for several years, and many will not develop symptoms until adult life, if at all. This is thought to be due to the predilection of the primary complex for the upper lobes, so that bronchiectasis in this situation remains "dry" because of self-drainage.

The factor responsible for the production of bronchiectasis is bronchial obstruction by a tuberculous gland, whether by extrinsic pressure or by erosion through the bronchial wall with the development of endobronchial granulations. Tuberculous infection of the bronchial tree probably also plays a part, but only in conjunction with the obstructive element. At least 5.9 per cent of all established bronchiectatics with symptoms are considered to have primary tuberculous etiologies.

M. J. SMALL

Bronchography in Pulmonary Tuberculosis. T. F. MCCARTHY* and D. P. MISRA. *Brit. J. Dis. Chest*, July, 1959, 53: 319–323. (* Stockport Clinical Area, England)

Since modern chemotherapy of tuberculosis has minimized the risk of spread of disease after bronchography, and newer contrast media no longer obscure the roentgenographic appearances, it was decided to carry out bilateral bronchography on a series of tuberculous patients in sanatorium. The purpose was to decide the place of the procedure in assessing treatment and prognosis, and to correlate the roentgenographic and bronchographic appearances with the pathologic findings in the resected specimens. The method used was to introduce propyliodine (Dionosil®) via the cricothyroid membrane under local anesthesia. No case of spread of disease was encountered and there were no significant complications of the method, although a mild febrile reaction lasting 3 to 4 days occurred quite commonly. Bronchiectasis in tuberculosis was most commonly encountered in those segments which also frequently showed bronchial crowding due to collapse and fibrosis. Extensive parenchymal disease was accompanied by gross bronchiectasis, and minimal disease by normal bronchograms. Bronchiectasis was found in 68 per cent of cases.

Since the place of surgery in pulmonary tuberculosis has become smaller and more accurately defined and since greater reliance is being placed on long-term chemotherapy, in which bronchography is of little value in the assessment of prognosis, routine bronchography in all cases is no longer indicated. Certain indications, however, remain

for its use. Prior to surgery, bronchography will demonstrate the association between the parenchymal disease and the bronchi and, by excluding bronchiectasis unsuspected by roentgenography or tomography, assist in the decision as to the type and extent of the surgery. It may show bronchiectasis when hemoptysis occurs in seemingly inactive disease, or when apparent control of the tuberculous parenchymal disease has been obtained and when symptoms, especially cough and sputum, persist in spite of chemotherapy.

M. J. SMALL

Erroneous Clinical Diagnosis of Tuberculosis and Its Consequences (in German). G. ENDRES*. *Ztschr. Tuberk.*, July, 1959, 113: 197–200. (*Medizinische Akademie, Erfurt, East Germany)

An unspecific pneumonia in a 15-year-old girl was diagnosed as pulmonary tuberculosis on the right side. The roentgenographic findings on the left side were interpreted as burnt-out tuberculous lesions. The patient was treated for years as tuberculous. Her professional schooling was interrupted. Only 7 years later was the diagnosis corrected: the left lung was hypoplastic, resulting from displacement of abdominal viscera into the left thorax, which was caused by malformation of the diaphragm. The patient has since been well and is fully able to work.

J. HAAPANEN

A Clinical Study of the Haemagglutination Reaction in Tuberculosis. J. SPÄNBERG*. *Acta tuberc. scandinav.*, Supp. 46, 1959: 1–96. (*University Hosp., Uppsala, Sweden)

Clinical investigation of the Middlebrook-Dubos hemagglutination reaction was carried out on patients with active and inactive pulmonary tuberculosis, extrapulmonary tuberculosis, sarcoidosis, and nontuberculous diseases, and in healthy individuals. Carried out according to the methods used in this investigation, the hemagglutination reaction has very limited value as a diagnostic aid in tuberculous infection. The reasons are these: (*1*) The method error is so great that only a difference of 3 titer steps can be accorded significance. Method error of such magnitude leaves doubt as to the result of the reaction and probably requires double or triple determinations for judging isolated samples with safety. (*2*) The reaction shows an inadequate power of selection. Positive reactions are not uncommon in nontuberculous individuals and negative reactions are common in persons with tuberculosis. (*3*) False positive reactions occur in sarcoidosis to the same degree as in tuberculosis.

Although the results are better with some antigens than with others, there has been no success in obtaining a clinically useful result with any antigen from tuberculin or bacillary bodies. The large method error makes the reaction completely unserviceable as a "check-up reaction" in the course of tuberculous disease. No conclusions can be drawn from the reaction as to the immunologic condition or the prognosis in tuberculosis. The reason for the failure of the reaction is probably that, in tuberculosis, immunity is not principally related to a circulating antibody but exists on the cellular plane.

W. J. STEININGER

Lymph Node Perforation through the Bronchial Tree in Children with Primary Tuberculosis. S. FROSTAD*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 104–124. (*Grefsen Sanatorium, Oslo, Norway)

Lymph node perforation is a relatively frequent occurrence in Norwegian children with primary tuberculosis; it was observed in 36 (24.7 per cent) of 146 children routinely bronchoscoped. Perforation occurs more frequently: (*1*) in small children below six years of age, (*2*) within the first half year after conversion of the tuberculin reaction, and (*3*) on the right side of the bronchial tree, the region of the right stem bronchus being the site of predilection. It appears to occur in spite of antituberculosis chemotherapy.

Roentgenographically, cases of lymph node perforation most often show atelectasis in one or more lung segments, but the perforation may occur without visible lung affection and without visible hilar adenitis. After perforation the shadow shows regression, but only if the bronchial occlusion has not persisted for too long. Perforation is lacking in symptoms. Loss in weight or failure to gain, together with sudden clearing of an atelectatic zone, provide strong suspicion of lymph node perforation. Atelectasis and bronchiectasis may persist, however. Since antimicrobial therapy of perforation is unsatisfactory, it is reasonable to seek other methods of treatment. Whether this should consist of surgical glandular toilette, roentgen therapy, or corticosteroid therapy, only the future will show.

W. J. STEININGER

Extraperiosteal Plombage in the Treatment of Pulmonary Tuberculosis (in Greek). C. SCOUTELIS*. *Acta chir. hellenica*, May–June, 1959, 6: 275–298. (*Sismanoglion Institute, Athens, Greece)

The series presented comprises 25 operations in 24 patients from September, 1953 to August, 1955. Twenty patients had bilateral disease, and 12, severely impaired pulmonary function or cardio-

vascular disease. Seventeen patients were between 23 and 37 years of age, 6 between 35 and 50, and 1 was 70. Polythene spheres were used in 14 cases, polystan in 9, and polystan spheres in 2. The results were analyzed from 31–52 months after operation.

There was no mortality. Immediate postoperative space infection occurred in 1 case. Late space infections were observed in 2 within about 1 year after plombage operation. These cases were successfully treated by removal of the plombe and addition of a seven-rib thoracoplasty. In 18 cases the sputum cultures became negative and tomograms showed no cavity. In the 6 other patients, in whom sputum remained positive, 2 had cavity closure under the plombage but contralateral cavity or active disease. In 2 cases plombage failed to close the cavity, and in the other 2 no cavity could be demonstrated by tomography. The operation is supplemental to, rather than competitive with, resection. It is preferable to thoracoplasty and is indicated when resection is not feasible because of poor cardiorespiratory reserve.

N. C. Laios

TUBERCULOSIS, NONPULMONARY

Ophthalmic Primary Infection (in Portuguese). B. C. Lima*, C. Frazatto, Jr., and F. J. Salles. *Rev. paulista tisiol.*, May–June, 1959, 20: 263–268. (*Santa Casa de Misericórdia, Campinas, São Paulo, Brasil)

Among 219,584 patients seen between 1935 and 1957 at an Institute of Ophthalmology (Institute Penido Burnier, Campinas), 29,524 had conjunctivitis and 2,396 dacryocystitis. There were only 3 cases of ophthalmic primary tuberculosis: 1 among the patients with conjunctivitis and 2 among those with dacryocystitis.

J. F. Carneiro

Contribution to the Question of Tuberculosis during Pregnancy and Delivery (in German). P. Schwarz*. *Wien. klin. Wchnschr.*, September 4, 1959, 71: 677–679. (*Universitäts-Frauenklinik, Innsbruck, Austria)

Between the years 1948 and 1958, 30 female patients with tuberculosis were observed during pregnancy and delivery. Of these, 23 had pulmonary tuberculosis, 5 had tuberculous meningitis, 1 had tuberculous coxitis, and 1 had undergone a pneumonectomy. During this period no pregnancy was interrupted because of tuberculosis. One patient with pulmonary tuberculosis and 1 patient with tuberculous meningitis died after their deliveries; both patients had been treated too late and insufficiently. In none of the children whose mothers had tuberculous meningitis could tuberculosis be found. No tuberculosis was found in the 2 placentae which were examined histologically. Interruption of pregnancy because of tuberculosis is not indicated, with rare exceptions. A gentle method of delivery, especially shortening of the second stage, is recommended. Early and long-lasting chemotherapy in a sanatorium is essential.

G. C. Leiner

Renal Tuberculosis in a Native Population. W. E. Beamish*. *Canad. M. A. J.*, August 15, 1959, 81: 238–241. (*Charles Camsell Hosp., Edmonton, Alberta, Canada)

Among 6,559 patients admitted to the Charles Camsell Hospital between 1946 and 1958, 104 (1.6%) were found to have renal tuberculosis. The cases were equally divided between males and females. Renal tuberculosis was found to be associated with pulmonary tuberculosis in 51 patients (49%), with pleurisy in 13 (12%), with bone and joint tuberculosis in 32 (30%), with genital tuberculosis in 19 (18%), with meningitis in 2 (1.9%), and with pericarditis in 1 (1%). Sixty-nine patients (66%) had no symptoms; 8 patients (7.7%) had only 1 symptom. Gross hematuria occurred in only 9 patients (8.7%). Frequency was the most common symptom. None of the patients had normal urine on routine analysis, but grossly abnormal findings were unusual. In 27% there were less than 10 pus cells per high-power field, and no red cells. Among 92 cases studied by intravenous pyelograms, 11 were normal, 14 were only suspicious, and 67 were definitely abnormal. Fifty-six patients (53.8%) had unilateral disease only. All patients had chemotherapy with various combinations and 29 underwent nephrectomy. The average hospital stay for 29 patients under treatment for genitourinary tuberculosis alone was 16.7 months, and all had at least 6 months' drug treatment after the last positive urine culture. There were 6 deaths in the series, but only 1 was due to renal tuberculosis. Among 34 patients followed for more than 6 months, 5 were found to have a bacteriologic relapse. Of the 29 nephrectomized patients, only 4 were operated upon after isoniazid became available. Triple-drug therapy is now recommended for a minimum of 18 months.

E. A. Riley

THORACIC SURGERY

Agenesis of the Left Lung with Diaphragmatic Relaxation: Report of a Case (in Japanese). S. Kimura*, N. Tekiba, T. Ikemori, and Y. Numazaki. *Jap. J. Thoracic Surg.*, September, 1959, 12: 753–756. (*Tohoku U., Sendai, Japan)

A case of agenesis of the left lung with diaphragmatic relaxation is reported. The patient was a

32-month-old girl who suffered from recurrent vomiting of a half month's duration. Relaxation of the diaphragm was diagnosed roentgenographically, but thoracotomy revealed a complete absence of the left lung. Reinforcement of the left diaphragm and left thoracoplasty were carried out on this patient, and her complaints disappeared completely following surgery.

The same disease has been reported 10 times previously in Japan. Nine of the cases were confirmed at autopsy, and 1 was diagnosed roentgenographically. Eight were less than $2\frac{1}{2}$ months old, but 1 case diagnosed by film was 13 years old and was still alive, while another case was a 64-year-old male who died of stomach cancer. Up until 1953, 73 cases of agenesis, 38 cases of hypoplasia, and 4 cases of bilateral aplasia of the lung were reported in the European and American literature.

I. TATENO

Bronchoscopic Findings in Exudative Pleurisy in Children and Adolescents (in German). H. DOESEL*. *Tuberkulosearzt*, July, 1959, 13: 453–459. (*Kinderheilstätte Wangen im Allgäu, Germany)

Bronchoscopy was performed in 100 children and adolescents who had developed exudative pleurisy in the course of primary pulmonary tuberculosis. The acute stage of the disease was always accompanied by inflammation of the bronchial mucosa of the affected side. In some cases the bronchi of the contralateral side were also involved, although to a lesser extent. The inflammatory reactions were most pronounced in those portions of the bronchial system which were in open communication with caseating lymph nodes, and in bronchi draining secondary foci. Histologic examination of biopsy specimens revealed chronic inflammatory processes in the subepithelial tissue with a preponderance of lymphocytes, histiocytes, and plasma cells. Inflammatory atrophy of the bronchi was present in 9 per cent of the cases. In 25 per cent, erosion of the bronchus by caseating lymph nodes had occurred and a fistula was present. The sputum was positive for *M. tuberculosis* in 18 cases. Fistulas and scars were found more often on the right than on the left. Deformation of the bronchial tree due to scar formation was found in 3 per cent of the cases.

J. HAAPANEN

Removal of Cervical Ribs (in German). K. Jós* and Á. PAPOLCZY. *Ztschr. Tuberk.*, July, 1959, 113: 141 – 146. (*János - Spital, Budapest, Hungary)

In this new modification of surgical removal of cervical ribs, the skin incision follows the anterior border of the trapezius muscle in the direction of the cervical rib. With this method the surgeon can immediately obtain easy access to that part of the rib which is relatively free of other tissues. Once this part is liberated, it is easy to free the whole rib.

J. HAAPANEN

Effect of Decortication on Pulmonary Function in Patients with Pyothorax (in Japanese). M. TAKEI*. *Jap. J. Thoracic Surg.*, September, 1959, 12: 792–808. (*Chiba U. School of Med., Japan)

The effect of decortication on pulmonary function in 32 cases of pyothorax was analyzed. Pulmonary function tests were made about one year after operation and the following conclusions were obtained. (*1*) Decortication is superior to other collapse therapy in tuberculous pyothorax from both the clinical and functional points of view. (*2*) Improvements in pulmonary function depend upon intrapulmonary lesions: when they are localized and minimal, functional improvement may be expected even in tuberculous pyothorax. Reexpansion of the lung and improvement in pulmonary function cannot be expected in those with active pulmonary lesions requiring treatment. Thoracoplasty may be required in these cases and, when the pulmonary lesions are extensive, decortication is impossible and pleuropneumonectomy may be indicated. (*3*) Improvement in pulmonary function corresponds with roentgenographic reexpansion of the lung and was reflected by the increased ventilatory function and oxygen uptake by the diseased lung.

I. TATENO

Experimental Use of a Diaphragmatic Flap in the Plastic Repair of Injuries and Resected Myocardial Infarcts (in Russian). S. M. LUTSENKO*. *Khirurgija*, August, 1959, 8: 17–24. (*Kishinevski Med. Institute, U.S.S.R.)

Methods and results of experimental work on dogs are presented. A diaphragmatic flap is used to establish collateral circulation and repair defects of the myocardium. Angiographic studies of the diaphragm showed a rich anastomosing blood supply. Flaps on a pedicle are cut 6–9 cm. long, 3.5–4.5 cm. wide. The thickness of the muscle flap may be split in half from the pedicle to within 1.5 cm. of the free border, and thus the length of the flap is nearly doubled. The diaphragmatic flap was sutured to the myocardia of 13 dogs. Electrocardiography, kymography, angiography, and thoracotomy at various intervals failed to show any limitation of muscular contraction. The flap had grown firmly to the heart wall; an intimate blood supply became established within 2–3 weeks.

Five dogs had the lower third of the anterior

branch of the left coronary artery tied off. The ischemic portion of the heart muscle was excised and the defect sutured over with a diaphragmatic flap. In 10 dogs the ischemic heart muscle was covered with a diaphragmatic flap without excision. Five dogs had stab wounds through the myocardium of the left ventricle sutured over with a diaphragmatic flap. The postoperative course of all of the animals was stormy. Fibrillations, extra-systoles, pneumonia, pleural exudates, and empyema were the complications observed. Of the dogs that had resection of the myocardium, 3 died the first week. Of the 10 dogs that did not have resection, 6 survived. Of the 5 that had the stab wounds, 3 died.

In examination of the dogs that died and those that were sacrificed at various intervals later, groups one and three showed no evidence of blood leakage at the operative site. The diaphragmatic flap was densely adherent in all dogs, and the edges of the wound were sealed. Deposition of fibrin fused the borders of the flap to the myocardium. Later stages showed connective tissue bridging the flap and myocardium. The flap retained the same thickness, retained its reddish-rose color, and showed no outward bulge. The ischemic myocardium that was not excised showed revascularization from the blood supply of the flap. The inner surface of the myocardium that had the stab wounds showed thrombi. Histologic studies showed solid union between the myocardium and the flap, characterized by vascular proliferation and a dense network of connective tissue.

W. A. Zavod

Results of Pneumonectomy in Japan (in Japanese). T. Wada* and T. Tsuchida. *Jap. J. Clin. Tuberc.*, September, 1959, 18: 595–606. (*Sapporo Med. College, Japan)

Analysis was made of 1,801 cases of pneumonectomy performed in Japan. The number of pneumonectomies steadily increased from 1953. The operation was done against tuberculosis, malignancies, infections, bronchiectasis, and benign neoplasms in 1,245, 234, 85, 53, and 4 cases, respectively. Complications of the operation in pulmonary malignancies and infections occurred in 27 per cent, but they were very low in bronchiectasis, and the death rate was highest in the first group.

The rate of performance of thoracoplasty, lobectomy, and pneumonectomy in recent years was 1:8:1, indicating a remarkable decrease in thoracoplasties. Pneumonectomy for tuberculosis was done more frequently in males, on the left side, and in patients in their 20's, 30's, and 40's, in that order. The patients had been treated internally for more than 3 years in most cases before they underwent surgery. Postoperative complications were seen in 30 per cent, and thoracoplasty was necessary in about the same percentage. Drug-resistant tubercle bacilli and contamination of the thoracic cavity during operation were considered responsible for the development of postoperative complications. These complications were cured in 90 per cent 1 to 2 years after operation. The death rate was about 10 per cent; most died early, and pulmonary insufficiency and hemorrhage were the responsible causes in most cases.

I. Tateno

Pulmonary Resection for Tuberculosis in Children. J. L. Hansen*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 143–151. (*Oresund Hosp., Copenhagen, Denmark)

During an 8-year period, 12 pulmonary resections were performed for tuberculosis in children. During the same period, pulmonary resections for nonspecific chronic infections in the same population (Copenhagen children) were 4 times as frequent and, for tuberculosis in adults, 100 times as frequent. Pneumonectomies gave unsatisfactory results (50 per cent recoveries), whereas bilobectomies and lobectomies in all cases resulted in apparently final recovery. No segmental resections were performed. Sequelae of pulmonary tuberculosis such as bronchiectasis, bronchostenosis, pneumonitis, nonspecific abscess, and vascular shunting were more often the indications for pulmonary resection for tuberculosis in children than was the tuberculosis itself.

W. J. Steininger

Surgical Treatment of Pulmonary Echinococcosis (in Russian). Z. M. Dagdzhi*. *Khirurgija*, August, 1959, 8: 86–89. (*Samarkand Med. Institute, U.S.S.R.)

Echinococcal disease is common in Uzbekistan. During 1950–1957, the Samarkand Medical Institute treated 25 cases of pulmonary echinococcosis with resection. The cysts were bilateral in 2 cases. All of the patients had single-stage operations. The cysts when exposed were first injected with 5 ml. of 2 per cent formalin solution. Twenty-four patients recovered uneventfully with the aid of antimicrobials, blood transfusions, and oxygen postoperatively. One patient had to be reoperated upon. There was no postoperative mortality.

W. A. Zavod

Operability and Results of Resection in Cancer of the Lung (in Russian). S. I. Danilenko*. *Khirurgija*, August, 1959, 8: 79–82. (*Vishnevsky Institute of Surgery, AMN, U.S.S.R.)

From 1950 to 1957, 612 cases of cancer of the lung were registered at the Vishnevsky Institute: 548

(89.5 per cent) were men and 64 (10.5 per cent), women. The right lung was involved in 424 (69.2 per cent); the left, in 188 (30.8 per cent). Initial examination disclosed that 324 (53 per cent) were too far advanced to consider surgical intervention, while 288 (47 per cent) were hospitalized for study and treatment. Of the latter, 118 (19.3 per cent) were found after study to be too far advanced for thoracotomy. The remaining 170 (27.7 per cent) were explored. Of these, 89 (14.6 per cent) were not resectable. Lobectomy or pneumonectomy was done in 81 (13.1 per cent) of the patients.

Analysis of comparable cases that were explored with those that were not explored showed that thoracotomy shortens the life of the patient. Despite that, the author advises exploratory operation since inoperability cannot be established on the appearance of the roentgenogram alone and in cases in which the diagnosis of cancer has not been definitely established. Follow-up records are available on 53 cases of the 81 that had radical resections. Only 9 survived 5 years. Of these, 7 had squamous cell carcinoma. Tying off the pulmonary artery in 14 unresectable cases did not prolong life. Undifferentiated carcinoma had the worst prognosis. Involvement of the lymph nodes of the root of the lung had a better prognosis than the cases with involvement of the mediastinal lymph nodes as well.

W. A. Zavod

PUBLIC HEALTH AND EPIDEMIOLOGY

Occupational Lung Cancer: A Review. R. Doll*. *Brit. J. Indust. Med.*, July, 1959, 16: 181–190. (*Medical Research Council, London, England)

There are five established risks for occupational lung cancer in Britain: radioactivity—as in the mining of heavy metals contaminated with radioactive elements, nickel, chromates, asbestos; and gas production, as in the combustion and distillation of coal products. In addition, there are several suspected risks for occupational lung cancer. These include arsenic, iron, isopropyl oil, and beryllium. Of interest also is the apparent absence of occupational cancer in certain industries in which a high incidence of cancer might be expected *a priori*. For instance, consider industries in which the employees have a high exposure index to fumes of petrol or diesel-driven engines. These fumes may contain measurable amounts of several polycyclic hydrocarbons. Nevertheless, workers so exposed seem on analysis actually to have a lower incidence of lung cancer than open-air workers of the same age group.

The second group of industries concerns those with a hazard of silicosis. Here again, the prevalence of lung cancer at necropsy was lower than in comparable groups of necropsies obtained on people not exposed to silicosis. It is emphasized that an industrial hazard of lung cancer depends on the specific characteristic of the industry, and not on any nonspecific effect of pneumoconiosis. (Transport workers and coal miners do experience significant degrees of pneumoconiosis in the pursuit of their occupations.)

Interestingly enough, there is a certain site-specificity in the development of occupational lung cancer. The risk is usually confined to the lungs or, at the most, one other site, i.e., nose cancer in nickel workers and in the chromate industry. Lung cancer is not associated with laryngeal cancer in any of the industries studied. The reasons for this are not understood, but speculation centers around genetic considerations, or the possibilities of organ-determined differences in susceptibility to neoplasia. All forms of industrial lung cancer are characterized by a very long latent period, averaging 15 to 30 years after first employment.

H. Simon

Bronchogenic Carcinoma: A Mass Radiography Group Compared with a Practitioners' Group. J. Cuthbert*. *Brit. J. Dis. Chest*, July, 1959, 53: 217–225. (*Chest Clinic, S.E. Glasgow, Scotland)

Forty-eight cases of bronchogenic carcinoma seen at one clinic in Glasgow after the city-wide mass roentgenography survey are compared with 48 consecutive cases with symptoms sent to the clinic by practitioners. Only 12 of the survey group had no symptoms, and it is concluded that more propaganda is necessary to make the public conscious of the importance of roentgenography in conjunction with chest symptoms.

On the whole, the survey group fared better than the practitioners' group and, at 18 months from first attending the clinic, 13 of them were alive, compared with 8 in the practitioners' group. Difficulties of diagnosis arose with those cases which presented as pneumonia, peripheral abscess, or with lymphangitis carcinomatosa, resembling tuberculosis on the roentgenogram, and those with negative bronchoscopic findings.

Operation, be it removal of one lobe or pneumonectomy, offers the best hope of survival to 18 months. It appears that, in order to get cases of carcinoma early enough to be treated, one would have to film persons in the cancer age group approximately every 6 months.

M. J. Small

Influenza 1959. J. Fry*. *Brit. M. J.*, August 8, 1959, No. 5144: 135–138. (*Beckanham, Kent, England)

Among 6,016 cases of influenza seen in a general practice during the winter of 1959, the incidence

of pulmonary complications (9.5 per cent) was higher than in any previous epidemic. There were 7 cases of lobar pneumonia, 43 of segmental pneumonia, and 30 cases of acute bronchitis. Only 6 of these patients had underlying lung disease. The age distribution of these complications showed the highest rates in the young and the old. Sputum examination did not reveal any predominant organisms. No staphylococci were detected. The moderately ill patients received penicillin, but it was found necessary to use broad-spectrum antimicrobials in addition in the most severely ill patients. Response to antimicrobials was often very slow.

E. A. RILEY

Asian Influenza in High School Students. K. D. ROGERS* and H. M. GEZOU. *Pub. Health Rep.*, October, 1959, 74: 925–935. (*Graduate School of Public Health, U. of Pittsburgh, Pa.)

The etiology of an epidemic of acute influenza-like illness in Allegheny County, Pa. in the fall of 1957 was investigated. High-school students were questioned concerning symptomatology of any illnesses during this time period, and complement-fixing antibody tests against the Asian variant of influenza type A were done with blood samples from part of this group. The signs and symptoms reported most commonly with illness during this period (sensitivity), were combined with the signs and symptoms most frequently present in patients exhibiting changes in titer of specific antibody (specificity). The combined sensitivity-specificity index was used to construct a clinical syndrome considered appropriate for estimating incidence of specific illness during an epidemic period.

E. DUNNER

South American Blastomycosis (in Portuguese). A. S. ALMEIDA*, A. TRAMUJAS, S. M. MACHADO, and H. BRANDÃO. *Rev. paulista tisiol.*, May–June, 1959, 20: 209–218. (*Sanatório Médico Cirúrgico, Portão, Paraná, Brasil)

Sixteen cases of Lutz's disease (South American blastomycosis) are presented. In 3 cases the patients had both pulmonary blastomycosis and pulmonary tuberculosis.

J. F. CARNEIRO

Epidemic Histoplasmosis. G. L. BAUM*, J. SCHWARZ, and H. FLOYD. *Tuberculology*, September, 1959, 18: 30–33. (*V.A. Hosp., Cincinnati, Ohio)

Histoplasmosis is acquired in the endemic area by inhalation of spore-containing dust. In special circumstances in which the atmosphere becomes oversaturated with spores, a special clinical picture occurs which consists of multiple primary foci in the lung. The majority of cases recover. Examples of miliary densities in the lung are presented, one proved by biopsy, another at autopsy.

E. DUNNER

Silicosis in Laisvall (in Swedish). L. NORVIIT*. *Nord. med.*, July 16, 1959, 62: 1076–1081. (*Boliden, Sweden)

The mined ore at the Laisvall lead mines contains 80 to 90 per cent quartz. During the first 7 years of operation, the dust concentration in the air of the working areas was much more than 1,000 particles per ml., and the incidence of silicosis was 45 per cent of 76 exposed persons. During the last 5 years, the density of the dust particles had been reduced to 100 to 200 per ml. and there was not one case of silicosis diagnosed among 103 exposed persons.

J. HAAPANEN

Unusual Manifestations of Silicosis. C. S. MORROW*. *Tuberculology*, September, 1959, 18: 35–38. (*V.A. Hosp., Wilkes-Barre, Pa.)

Two unusual cases of silicosis are presented: a patient with silicotic pneumolithiasis and another with Caplan's syndrome.

E. DUNNER

Smoking Habits of Massachusetts Physicians: Five-Year Follow-up Study (1954–59). L. S. SNEGIREFF* and O. M. LOMBARD. *New England J. Med.*, September 17, 1959, 261: 603. (*Harvard School of Public Health, Boston, Mass.)

A marked change has occurred in the smoking habits of Massachusetts physicians during the 5-year period from 1954 to 1959. The percentage of physicians who abstain from smoking has risen from 34 to 45. Simultaneously, a decrease has been evident in the number of physicians who smoke cigarettes. This decrease is very apparent among those who smoke 1 or more packages of cigarettes per day. Ninety-three per cent of the physicians stated that they believed "Massachusetts teen-agers should be informed of the potential danger of smoking cigarettes as an avenue that may lead to lung cancer in later years." Many physicians stated their opinion that teen-agers also should be informed of other potential hazards of cigarette smoking, such as generalized effects on the circulatory system and local effects on the respiratory system, including chronic pulmonary disability.

Objections were made to the methods employed in advertising by the tobacco industry, and the frequent use of cigarettes in movies, on television, and the stage was criticized. The consensus of the majority of members of the Massachusetts Medical Society indicates that something should be done to warn teen-agers against smoking cigarettes. These data emphasize the importance of

the cigarette problem and the awareness of an alert medical profession.

M. J. SMALL

Studies of Pathogenic Staphylococci in the Upper Respiratory Tract of Members of Hospital Staff. T. M. VOGELSANG* and H. HAALAND. *Acta path. et microbiol. scandinav.*, No. 3, 1959, 45: pp. 281–308: (*Gade Institute, U. of Bergen, Norway)

I. Bacteriophage Typing: pp. 281–294:

The upper respiratory tracts of 845 members of the staff of the University Hospital in Bergen yielded 995 pathogenic staphylococcal strains which were examined for frequency of lysis by 22 different phage filtrates constituting the basic phage set: 88.8 per cent of the strains gave positive results by the phage method, 69.2 per cent by the Routine Test Dilution and, additionally, 19.6 per cent by employing phages at 1,000 times the concentration used in the Routine Test Dilution. Only 11.2 per cent of the staphylococcal strains were untypable by this method. On the basis of the lytic effect of the phages, the typable strains were classified into five main groups. The strains within the groups were further divided into different phage patterns. A few of the phage patterns within the different groups were regularly found in the clinical wards as well as in the non-clinical departments and must be regarded as local patterns for this hospital. Most of the phage patterns, however, were scattered among the staff, which suggests that they harbor pathogenic staphylococci as intermittent hosts in their upper respiratory tracts.

II. Phage Groups and Phage Patterns of Antibiotic Resistant Staphylococcal Strains: pp. 295–308:

From 845 members of the staff in the University Hospital in Bergen, 995 pathogenic staphylococcal strains were isolated from the throat and/or the nose. The resistance of these strains to sulfathiazole and 9 antimicrobials was compared with the phage typing of the same strains. Of the 92 strains resistant to sulfathiazole, $^2/_3$ belonged to phage group III and about $^2/_5$ to group I. Of the 369 strains resistant to penicillin, about $^1/_2$ were due to group III, $^1/_5$ to group I, and $^1/_{10}$ to a miscellaneous group, consisting mostly of overlapping between groups I and III. Of the 128 strains resistant to streptomycin, about $^2/_3$ were confined to group III, and about $^1/_3$ to group I. Of the 28 strains resistant to tetracycline, 23 belonged to group III. Of the 16 strains resistant to oleandomycin, chloramphenicol, or novobiocin, 11 were due to group III. None of the strains was resistant to erythromycin, neomycin, and nitrofurantoin. Of 151 straphylococcal strains resistant to more than one of the chemotherapeutic agents, 101 belonged to group III, 38 were due to group I, and a few strains were confined to other groups. Although there still is a great preponderance of resistant strains belonging to group III, in this study resistant staphylococci have been more common in group I and in the miscellaneous group than was found in earlier examinations. Resistant strains due to other groups are rarely identified. The strains in group III have a tendency to accumulation in a few patterns having as the main combination lysis by phages 6/7/47 or by phage 77. In group I the main combination is lysis by phages 52/52A or by phage 80.

E. DUNNER

Comparison of the Potency of the Japanese and International Standard Old Tuberculins (in Japanese). K. YANAGISAWA*, D. MAEDA, and N. ASAMI. *Kekkaku*, August, 1959, 34: 556–560. (*National Institute of Health, Tokyo, Japan)

A comparative study was made of the Japanese Standard Old Tuberculin (Jap. OT) prepared by the National Institute of Health of Japan, and the International Standard Old Tuberculin (Internat. OT) received in 1952 and 1957 from the State Serum Institute of Copenhagen. (*1*) The Internat. OT received in 1952 was less potent in animal experiments than was the Jap. OT prepared in 1947, but they were of equal potency when tested at 1:2,000 dilution in humans. (*2*) The Internat. OT received in 1957 and the Jap. OT prepared in 1953 were equally potent in animal experiments, but the former was 3 times more potent than the latter when tested at 1:2,000 dilution in humans. (*3*) The Jap. OT prepared in 1947 and 1953 were equally potent, but the Internat. OT had fluctuating potencies.

Incompleteness of the method of assay of the potency of the Internat. OT was suggested by Dr. Magnusson of Copenhagen. The importance of assaying the potency of OT both in man and in animals was reconfirmed. The establishment of internationally approved assay methods, and consistent potency of the International Standard Old Tuberculin are greatly to be desired.

I. TATENO

Current Problems on Tuberculin Reactions. II. How the Old Criteria of Positive Tuberculin Reactions Must be Modified (in Japanese). H. OKADA* and M. ASANO. *Jap. J. Clin. Tuberc.*, September, 1959, 18: 635–641. (*Nagoya U. School of Med., Japan)

Three methods of tuberculin testing are considered necessary in view of the changing patterns

of cutaneous tuberculin allergy in many individuals, especially in children who are BCG vaccinated by law and are frequently tuberculin tested by health officers in order to detect positive tuberculin conversions. When 1:2,000 OT is used as antigen, reading the tuberculin reaction at 24 hours is considered more practical in those who receive tuberculin tests often. Use of 1:100 OT in conjunction with 1:2,000 OT may be indicated in a limited number of patients, but this procedure will not be applicable in a mass survey. The use of PPD-S is another method in view of the specificity of the reactions caused by this material. However, the electrophoretic analysis of various PPD-S preparations indicated that the degree of purification differed between preparations made at different institutions, and further study in this direction was considered necessary.

I. Tateno

The Tuberculin Reaction in Female Hospital Patients. L. J. Taubenhaus*. *New England J. Med.*, August 13, 1959, 261: 328–330. (*Harvard School of Public Health, Brookline, Massachusetts)

Recent knowledge of the genetic and somatic effects of radiation has restricted roentgenographic examination as a mass screening device for detection of tuberculosis among the general population although its use is still recommended for populations of high risk. Hospital populations, when investigated, have been shown to yield a relatively high number of cases of tuberculosis. The tuberculin status of hospital populations has not been extensively studied. If a sufficient proportion of hospital patients are tuberculin negative, the tuberculin test can serve as an effective first-level screening device and eliminate the necessity of film examination of tuberculin-negative patients. What tuberculosis exists in a hospital population could then be found with fewer film studies, and radiation exposure could be further limited without a decrease in case-finding effectiveness.

All admissions to the Free Hospital for Women, Brookline, Massachusetts, between October 7, 1957, and September 30, 1958, who remained as inpatients over 48 hours were tuberculin tested. An attempt was made to give roentgenographic examinations to all patients who had positive tuberculin reactions and to a representative sample of those reacting negatively. The population encompassed in this study was considered representative of New England female surgical patients.

Of the 1,356 patients tested, 397 (30 per cent) had positive tuberculin reactions: 331 patients with positive tuberculin reactions were given film examinations. Of these, 203 (61 per cent) were negative for tuberculosis; 122 patients (37 per cent) had roentgenographic evidence of inactive tuberculosis, and 6 patients (2 per cent) had evidence of active tuberculosis.

On the basis of these findings, it is believed that the intradermal tuberculin test is an effective method for screening female surgical patients in the New England area.

M. J. Small

The Techniques of Tuberculin Testing. F. Heaf. *Acta tuberc. scandinav.*, Supp. 47, 1959: 153–161.

The historical development of the tuberculin test is reviewed briefly and a short description is given of the techniques now in use, together with a discussion of the advantages and disadvantages of each method. It is considered that the use of BCG tuberculin is worth pursuing in view of the many problems associated with the use of OT and PPD. A short reference is made to the comparative value of these two preparations. Further improvement of the tuberculin test is desirable in view of its increasing importance in epidemiologic work.

W. J. Steininger

Trial of a Self-firing Heaf Multiple-Puncture Machine. R. G. Carpenter* and C. J. Stewart. *Tubercle*, August, 1959, 40: 251–256. (*Dept. of Human Ecology, Cambridge, England)

A new type of multiple-puncture tuberculin testing machine, which is operated by a predetermined pressure being applied to the end plate, was designed to reduce the effects of variation in testing technique.

A trial comparing the new and standard Heaf multiple-puncture machines is described. This confirmed that the Heaf multiple-puncture test, whether done with the new or with the standard machine, can consistently classify subjects as positive and negative. The results obtained with the standard multiple-puncture machine showed that the effect of the tester on the size of the reaction had persisted for two years. The trial also showed that the pressure of the end plate on the skin at the moment of release had a decided effect with one tester on the size of the resulting reaction. The self-firing machine did not eliminate differences between testers in the size of reactions produced. It did limit the number of small reactions. The self-firing multiple-puncture machine is, therefore, thought to offer greater safeguards against tester error, but further study of the mechanics of the multiple-puncture technique is needed (Authors' summary).

M. J. Small

Appraising the Tuberculin Test. Editorial. *New England J. Med.*, August 13, 1959, 261: 355.

In this issue of the *Journal*, Taubenhaus presents data to support the value of the tuberculin test in screening asymptomatic female patients for pulmonary tuberculosis. Because this study was conducted in a woman's hospital in New England, the results may be difficult to apply to males and to other geographic areas. Nevertheless, all 6 cases of active tuberculosis were detected from among those reacting to 5 tuberculin units, and none of the 29 per cent of the subjects with negative reactions were found to have clinically significant pulmonary lesions.

There is no question that, when properly conducted, the tuberculin test can substitute effectively for a routine roentgenographic study of the chest. The loss of the more specific information about chest disease is more than offset by the avoidance of unnecessary cost and radiation exposure.

Five units (0.1 ml. of 1:2,000 OT) will not cause untoward reactions, and yet will still identify almost all patients with active tuberculosis. If over 100 units are used (over 0.1 ml. of 1:100 OT), reactions occur that are not related to infection by either the human or the bovine tubercle bacillus. In children the patch test is useful, but it is somewhat less specific than the intradermal injection of the Mantoux procedure.

M. J. SMALL

The Protective Value of Nonlyophilized and Lyophilized BCG Vaccine Against Tuberculosis. T. EBINA* and T. SAITO. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 162–174. (*Tohuku U., Sendai, Japan)

Comparative studies on the prophylactic and protective values of BCG vaccine were carried out among 3 different groups of persons. In the first group of 3,211 BCG vaccinated and 2,615 unvaccinated students, nurses, and inhabitants of 2 farm villages observed from 1 to 4 years, the morbidity from tuberculosis per 1,000 persons was significantly lower among the vaccinated. Among nurses observed for 4 years, the morbidity rate was 20.2 for the 198 unvaccinated, 14.4 for the 208 vaccinated. In a second group of persons attending an outpatient clinic because of symptoms suggestive of tuberculosis, the disease was observed approximately 4 times as often in 1,990 unvaccinated as among 2,147 BCG-vaccinated persons. More acute and more advanced disease was discovered among the unvaccinated individuals. Among the third group of 90 vaccinated and 282 unvaccinated patients hospitalized because of the serious nature of their tuberculosis, 99.2 per cent of the vaccinated and 77.7 per cent of the unvaccinated improved. The mortality from tuberculosis in all 3 groups was significantly higher among the unvaccinated. No significant differences were observed in the occurrence of tuberculosis and the type of disease among 252 who had received nonlyophilized BCG vaccine as compared with 378 who had received lyophilized vaccine.

W. J. STEININGER

Tuberculin Reactions and B.C.G. Vaccination in Old Age. D. M. PRINSLEY* and H. DROLLER. *Brit. J. Dis. Chest*, July, 1959, 53: 296–301. (*Middleton Hosp., Ilkley, England)

The chief problem of pulmonary tuberculosis now stands in relation to the elderly male. Various reasons have been advanced for this shift of the incidence of tuberculosis toward old age. However, some of these elderly patients have a roentgenographic appearance of acute disease of recent origin, and it has been suggested that this may be a manifestation of fresh infection in a tuberculin-negative subject. In order to discover the incidence of negative reactors to tuberculin among the elderly in this part of England, a tuberculin survey was carried out on a large number of patients in geriatric wards.

Tuberculin testing (by the multiple-puncture method) of 1,000 geriatric patients showed that there is no waning of tuberculin sensitivity with increasing age. It seems likely that some of the figures, quoted by various authors, which show declining sensitivity with increasing age are based on too small a number of patients. A variety of nontuberculous diseases (diabetes, arthritis, cancer, et cetera) among these patients did not affect the incidence of positive tuberculin reactions (67–80 per cent). Increased population density produces a higher percentage of positive reactors.

Using Danish liquid vaccine and British freeze-dried vaccine, 101 BCG vaccinations were carried out on negative reactors. The procedure in geriatric patients was found to be safe and effective in producing Mantoux conversion: 100 per cent conversion rate with the Danish liquid, and 92 per cent with the British freeze-dried vaccine.

M. J. SMALL

B.C.G. Vaccination—A Preliminary Communication. P. ELLMAN* and L. G. ANDREWS. *Brit. J. Dis. Chest*, July, 1959, 53: 302–307. (*East Ham Chest Clinic, London, England)

Few reports exist in England on the length of time tuberculin hypersensitivity and protection persist after BCG vaccination. This is a preliminary communication on 264 of more than 1,000 patients given Danish BCG from the State Serum

Institute in Copenhagen since 1950 and followed up for a period of 5 to 8 years. For a period of 5 to 8 years, 97.7 per cent maintained their hypersensitivity. This high figure may be partially due to the fact that 36 per cent of the cases had close contact with relatives known to have acid-fast bacilli in their sputum. To obtain more accurate figures for the length of time conversion lasts, vaccinated cases should ideally be segregated from patients with active tuberculosis. The incidence of proved primary tuberculosis despite persisting positive tuberculin tests was 0.39 per cent. This figure coincides with the results of previously published series. A further report will follow on the remainder of the cases when they have been observed for an adequate period.

M. J. SMALL

Sources of Tuberculin Sensitivity in Human Populations. A Summing Up of Recent Epidemiologic Research. L. B. EDWARDS*, P. Q. EDWARDS, and C. E. PALMER. *Acta tuberc. scandinav.*, Supp. 47, 1959: 77–97. (*U. S. Public Health Service, Washington, D. C.)

Disparities in the geographic patterns of sensitivity to small (5 or 10 TU) and large (100 or 250 TU) doses of tuberculin, and the fact that signs of tuberculous infection and disease are closely related to small-dose but not to large-dose sensitivity, point almost inescapably to the conclusion drawn 10 years ago that reactions to the 5-TU dose of tuberculin are largely a result of tuberculous infection, but most reactions to a large dose are cross reactions caused by infection with other organisms. Further studies have revealed that in some geographic areas many of the small reactions to 5 TU should also be interpreted as cross reactions; they appear to have the same significance as the sensitivity brought out more readily by the 100-TU or 250-TU test.

Comparative studies with standard tuberculin PPD-S and with PPD antigens prepared from various kinds of acid-fast organisms show that sensitivity to several of the new antigens is highly prevalent (many times more so than sensitivity to PPD-S) in some regions of the United States, notably the Southeast. Cross reactions to tuberculin apparently are the result of widespread infection with an organism (or organisms) more closely related antigenically to avian tubercle bacilli and some of the recently isolated "atypical" mycobacteria than to human tubercle bacilli. To improve the efficiency of preventive measures, a reliable method for distinguishing between specific tuberculin reactions and cross reactions is urgently needed. The most promising possibility lies in the development of appropriate antigens and techniques for skin testing.

W. J. STEININGER

Tuberculosis Mortality and BCG. Editorial. *New England J. Med.*, October 1, 1959, 261: 722.

Mass vaccination with BCG is generally opposed in countries in which, as in the United States, there has been a steadily declining mortality and morbidity resulting from other methods of control; indeed, there seems to be no great enthusiasm for its use even in the specially exposed groups. Modern methods of case finding, isolation, therapy, and chemoprophylaxis, properly applied with the aid of tuberculin testing, appear to be more useful and more successful in bringing ever closer the ultimate goal of the eradication of tuberculosis as a health problem.

M. J. SMALL

Tuberculosis Mortality and Morbidity and the Problem of BCG Vaccination in the United States. M. FINLAND*. *New England J. Med.*, October 1, 1959, 261: 699–702. (*Harvard Med. School, Boston, Mass.)

This paper is a review of recent literature on this subject. The majority opinion of the papers reviewed is summarized as follows: In diagnostic and case-finding techniques in children, the use of BCG vaccination and tuberculin testing are antagonistic procedures in the same community, and one plan or another, but not both, should be formally adopted as preferable. Tuberculin testing as widely used and recommended today should, it is believed, continue as the protocol of choice in tuberculosis case finding. Local communities, however, may decide in favor of BCG.

M. J. SMALL

Drug-Microbe-Host Mechanisms Involved in a Consideration of Chemoprophylaxis. W. MCDERMOTT*. *Bull. Internat. Union against Tuberc.*, July–October, 1959, 29: 243–258. (*Cornell U. Med. College, N. Y., N. Y.)

There is a considerable basis of scientific information relevant to the problems of both the primary and the secondary chemoprophylaxis of tuberculosis. From this body of information a case can be constructed both for and against the prophylactic procedure depending upon the weight one wishes to give to the various points. In this situation, with some conflict in the evidence, what practice should the clinician follow as a reasonable practice at the present time? It seems to the author that it can be stated that, because of the phenomenon of microbial persistence, it is not likely that primary chemoprophylaxis, i.e., the use of a drug to prevent the actual successful im-

plantation of the microbes, will prove to be a major component of tuberculosis control. This procedure would have a certain limited usefulness, however, in postponing for a matter of one or two years the time at which a young host, especially an infant host, might be required to face up to the implications of the primary infection. With respect to secondary chemoprophylaxis the advisable course seems much more clear. To be sure, in many cases, again because of microbial persistence, the administration of the isoniazid will be serving no useful purpose. By the same token, it would be doing no harm, other than the degree of harm involved in the risks of drug toxicity and the danger of the emergence of isoniazid-resistant infections. Although these two untoward happenings cannot be entirely discounted, the frequency of their occurrence would seem at this time to be so low as not to warrant a solid objection to the use of the procedure.

In sum, therefore, it seems wholly reasonable to advise today that *secondary chemoprophylaxis* with isoniazid be used in many persons who are tuberculin reactors. The particular tuberculin reactors who would most benefit from the procedure cannot yet be precisely identified but presumably include certain groups. Prominent among these are persons who either show some evidence of still having an active tuberculous lesion as, for example, by the size of the cutaneous reaction to tuberculin, or those whose age and circumstances make it likely that the infection was acquired within the preceding five- or six-year period. *Primary prophylaxis* could be used in highly specialized situations, such as the newborn and close family associates of persons with tuberculosis in an infectious stage. In these situations, however, the procedure should be integrated with the use of BCG either as a substitute measure, with the family associate group, or as a follow-up procedure, in the newborn of the tuberculous mother (Author's summary).

The Risk of Tuberculosis as a Guiding Factor for Tuberculosis Eradication. E. Groth-Petersen*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 132–138. (*Danish Tuberculosis Index, Svanemollevej 25, Copenhagen, Denmark)

Since the frequency and severity of tuberculosis varies in different segments of the population, a plea is made for more selective and intelligent expenditure of time, money, and roentgenograms in case-finding programs. Special attention should be directed to the following groups: (*1*) The fraction of the population more than 35 years of age. In one mass survey, more new cases of tuberculosis were discovered in men older than 35 than were normally found during an entire year by all usual methods (referrals for symptoms, military examinations, and routine examinations); in women more than 35, more than 1½ times as many. (*2*) Tuberculin-positive young people, particularly those with roentgenographic abnormalities other than clear-cut calcifications. Twelve per cent of this latter group develop active tuberculosis within 3 to 4 years. (*3*) All persons, regardless of age, with chronic roentgenographic lung findings of tuberculous or unknown origin. Persons older than 45 with such findings develop active disease 20 times more frequently than tuberculin-positive individuals with normal lung findings. Half of the adult population is tuberculin positive: 85 to 90 per cent of the new cases of tuberculosis will arise in that group. (*4*) Persons with symptoms or contact. Sixty-five per cent of new cases are found among the 12 per cent examined because of contact or symptoms. Just a single examination of healthy individuals more than 45 years old separates them into 2 groups: a small group who can and should be watched because of a high risk of developing tuberculosis, and a very large group who can be left in peace with themselves and their family doctor because their risk is so low.

W. J. Steininger

The Host in Tuberculosis. R. J. Dubos*. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 42–62. (*Rockefeller Institute for Med. Research, N. Y., N. Y.)

In the long run, the natural forces which affect resistance to tuberculosis are likely to prove more influential than any form of prophylaxis or drug therapy in determining the future course of the disease. The social environment affects the prevalence and severity of tuberculosis, not only by influencing the chances of contracting infection, but even more, perhaps, by determining the extent to which infection evolves into disease. Genetic determinants control many of the factors which influence the course of tuberculosis: the concentration of antibacterial substances in the body, the intensity of the inflammatory reaction, the type of response to the presence of bacilli.

The role of nutrition in tuberculosis, the mechanisms of acquired immunity, and the factors bringing about reactivation of arrested tuberculous processes are discussed. Nutritional intake of the proper amino acids, rather than the total amount of protein in the diet, is a determining factor of resistance. Diverse stimuli can activate or aggravate infectious processes, but they exert their effect through common pathways: paralysis of the reticuloendothelial system, increased proteolytic activity of plasma, and disturbances in secretion of steroid hormones. To the extent that chemoprophylaxis of tuberculosis is effective, it

will prevent the development of immunity. Virtual eradication of tuberculosis may return the population to a state of high susceptibility. It seems worth while to envisage a state of tuberculosis control based on the scientific philosophy that ways can be found to help man remain in ecologic equilibrium with the microorganisms ubiquitous in his environment.

W. J. STEININGER

Evaluation of the Tuberculosis Menace (in French). É. BERNARD*, Mlle A. LOTTE, and S. PRETET. *Rev. tuberc., Paris,* July, 1959, 23: 759–768. (*Paris, France)

From 1910 to 1958, the tuberculosis mortality in France has fallen from 215 to 24 per 100,000. However, in 1957 it was still the third highest in 13 European countries, exceeded only by the mortality rates in Spain and Portugal. The drop in mortality from 1930–1957 was 98 per cent from age 1 to 19 years, 98.3 per cent from 20–24 years, and 89 per cent during the first year of life. The drop in mortality declines with advancing age: it is only 47 per cent between 65 and 69 years. In 1930, 43 of 100 tuberculous deaths were female and 57 male; today the proportion is 28:72. Sixty-eight per cent of all tuberculous deaths occur in persons older than 50; 70 per cent of these are males. No reliable morbidity figures are available since tuberculosis is not a reportable disease in France.

V. LEITES

Rehabilitation of Tuberculous Patients in Finland (in German). J. PÄTIÄLÄ*. *Tuberkulosearzt,* July, 1959, 13: 492–500. (*U. of Helsinki, Finland)

The Influence of the Social Milieu on the Manifestation and Course of Tuberculosis. P. MUNTENDAM*. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 63–88. (*Ministry of Social Affairs and Public Health, The Hague, Netherlands)

Consideration is given to the influence of the social milieu on the manifestation and course of tuberculosis, with special regard to housing, deficient nutrition, lack of hygiene, ignorance, occupational surroundings, and psychic hygiene. Each factor is reviewed historically, and epidemiologic data are presented in assessing its impact upon the development and course of the disease.

W. J. STEININGER

Some Hazards in the Manufacture and Use of Plastics. D. K. HARRIS*. *Brit. J. Indust. Med.*, July, 1959, 16: 221–229. (*Imperial Chemical Industries, Ltd., England)

At high temperatures, polytetrafluorethylene gives rise to decomposition products that may be harmful when inhaled, and smoking contaminated tobacco is one of the commonest ways in practice by which workmen are affected. A general discussion of the use and possible toxicities of various plastic compounds is included in the discussion.

H. SIMON

The Acute Toxic Effects of Black Smoke. R. E. PATTLE*, G. D. WEDD, and F. BURGESS. *Brit. J. Indust. Med.*, July, 1959, 16: 216–220. (*Chemical Defence Establishment, Porton Downs, Salisbury, Wilts, England)

Few experiments have been conducted on the toxicity of solid carbonaceous combustion products. In order to obtain an estimate of the toxicity of black smoke which could be compared to similar estimates for other pollutants, the median dosage to death has been determined for smoke from burning tetrahydronaphthalene administered to guinea pigs and mice. Values from 147,000 mg. min/M^3 to 351,000 mg. min/M^3 were obtained at concentrations between 714 mg./M^3 and 1,140 mg./M^3. Animals allowed to associate protected one another to some extent, probably because their fur acted as a filter. In mice the cause of death appears to be a blockage of the air passages; deaths after removal from the smoke are unusual. In guinea pigs, hemorrhagic lesions are caused and delayed deaths are commoner. The action of smoke on rats resembles its action on mice.

It is pointed out that tetralin smoke is considerably more toxic to animals than is sulfur dioxide in the same concentration. This finding is discussed in relation to atmospheric pollution.

H. SIMON

ROENTGENOGRAPHY

Clinical Experience with High-Speed Electrons in Cancer Therapy. E. H. UHLMANN*. *Radiology,* July, 1959, 73: 76–84. (*Michael Reese Hosp., Chicago, Ill.)

Installation of a 45-Mev linear accelerator provided opportunity to utilize high-speed electrons (in the energy range of between 20 and 40 Mev) in the treatment of deep-seated malignant tumors. The principal difference between high-speed electrons and roentgen rays in the multi-million-volt range lies in their depth penetration which, in the case of electrons, is sharply defined and mainly dependent on the energy. Although the single-field technique is efficient in the treatment of properly selected patients, the possibility of using angular or opposite fields is an even more attractive feature of therapy with high-speed electrons. An important factor in the utilization of high-speed electrons is the relatively small volume of tissue that is subjected to irradiation,

even if the tumor is deep-seated. This factor alone may account for the absence of systemic reactions, often making it possible to carry out treatment on an ambulatory basis.

W. J. STEININGER

The Significance of Alterations in the Lung Arterial Pattern. W. H. SPRUNT*, R. M. PETERS, and D. L. HOLDER. *Radiology*, July, 1959, 73: 1–8. (*U. of North Carolina, Chapel Hill)

Venous and aortic angiography were performed in dogs after the pattern of blood supply to the lungs had been altered by transection and suture of a main bronchus, ligation of a main pulmonary artery, or by anastomosis of the left subclavian artery to the pulmonary artery (Blalock procedure). The experiments showed that it is possible to demonstrate the bronchial and pulmonary arterial circulation of the lungs with standard roentgenographic methods under conditions of atelectasis, pulmonary artery obstruction, and artificial shunt production. The information thus obtained will aid in the evaluation of pulmonary abnormalities, especially in cases in which surgical intervention is contemplated.

W. J. STEININGER

Hypoplasia of the Pulmonary Arteries. J. R. BELCHER*, L. CAPEL, J. N. PATTINSON, and J. SMART. *Brit. J. Dis. Chest*, July, 1959, 53: 253–262. (*London Chest Hosp., England)

Thirteen new cases of unilateral increased radiolucency of the lung (Macleod's syndrome) are reported. Bronchography, bronchospirometry, and angiography were carried out in the majority of the cases. All of the cases were shown to have small pulmonary arteries in the affected area. In 9, lobar arteries were involved, and in 4 the whole artery on one side was affected. In 2 cases the bronchograms were normal. In the remainder, the abnormality was not that of typical bronchiectasis. Bronchospirometry showed that the oxygen uptake was reduced considerably more than the ventilation on the affected side. The etiology is discussed. It is thought that the condition may be congenital in origin or that it may be acquired during the growing period. The abnormality does not cause symptoms, but it may aggravate breathlessness due to disease in the unaffected lung.

The diagnosis of the condition is essentially roentgenographic. The presence of hypoplasia of the pulmonary artery should be suspected whenever a lobe or lung shows increased radiolucency and diminished size. A close study of the vascular pattern on the postero-anterior chest film will usually establish the diagnosis. Angiography is helpful in doubtful cases, especially if there is difficulty in distinguishing between hypoplasia and congenital absence of the artery to one lung, although in practice the functional difference is often slight.

If there is effort intolerance and severe trapping in the abnormal lung, then pneumonectomy may help. This was carried out in 2 patients. The improvement in one was great, while in the other it was slight. In neither case was there any significant change in the spirometry findings after pneumonectomy. This reflects the severe ventilatory defect of the removed lung.

M. J. SMALL

Radiologic Signs of Pericardial Effusion. An Experimental Study. M. Z. MELLINS*, P. KOTTMEIER, and B. KIELY. *Radiology*, July, 1959, 73: 9–17. (* State U. of N. Y., Downstate Medical Center, Brooklyn, N. Y.)

Opaque pericardial effusions were produced in dogs by the direct injection of 70 per cent iodopyracet (Diodrast®) into the pericardial cavity. The animals were examined in the recumbent, erect, and four decubitus positions. The nature of the pulsations was recorded on roentgen kymograms. The fluid collected anteriorly, superiorly, laterally and, to a lesser degree, inferiorly. There was almost no posterior localization of the pericardial fluid. Furthermore, the location of the effusion was not altered to any appreciable degree by a change in position of the animal. The pulsations of the anterior pericardial surface are damped in pericardial effusion, while there is good transmission of cardiac pulsation to the barium-filled esophagus. In myocardial dilatation, the pulsations of both surfaces are roughly equal. Early clinical trials support the experimental findings.

W. J. STEININGER

Roentgenographic Diagnosis of Lesions Adjacent to the Large Fissure. Importance of Oblique Posterior Tomography (in French). P. K. SHINOHARA*, K. SANO, T. KOMORI, and M. SUMI. *Rev. tuberc., Paris*, July, 1959, 23: 740–757. (* Tokyo, Japan)

While the visualization of parts located posteriorly and inferiorly to the large fissure is often easy and satisfactory with postero-anterior and lateral tomography, the study of the middle and upper regions located posteriorly to the large fissure is less satisfactory, even with lateral tomography. Oblique posterior tomography at an angle of 50–60° should be used for the visualization of these regions which are so often the elective site of tuberculous and nontuberculous lesions.

V. LEITES

The Solitary Circumscribed Pulmonary Lesion Due to Bronchogenic Carcinoma: A 3 Year Follow-up Study of 94 Surgically Treated Patients. J. W. VANCE*, C. A. GOOD, C. H. HODGSON, J. W. KIRKLIN, and R. P. GAGE. *Dis. Chest*, September, 1959, 36: 231–237. (* Mayo Clinic, Rochester, Minnesota)

From 1944 through 1954, a total of 94 patients who met the following criteria were operated upon at the Mayo Clinic: (*1*) The lesion was solitary, situated in the substance of the lung, was more or less round, and had a more or less well-defined border. (*2*) The lesion was not associated with peripheral obstructive changes, central cavitation, or pleural effusion. (*3*) The diagnosis of bronchogenic carcinoma was made on the basis of pathologic examination of the excised tissue.

The resectability rate was 90 per cent. Twelve patients had palliative procedures (when it was apparent at operation that not all of the diseased tissue could be excised). This left 82 patients who had resections for what appeared to be all of the evident carcinoma, giving a "resection-for-cure" rate of 87 per cent. The hospital mortality rate was 5.3 per cent. The over-all 3-year survival rate based on 93 traced patients was 36.6 per cent. The 3-year survival rate for patients who had excision of all apparent bronchogenic carcinoma was 44.7 per cent.

The presence or absence of thoracic symptoms seemed to be the most significant prognostic variable studied; those without symptoms had a 15 per cent higher survival rate than those with symptoms. There was no significant difference in the survival rate whether pneumonectomy or lobectomy was the operation performed.

E. A. ROUFF

Bronchography with Barium in Patients with Pulmonary Tuberculosis (in Portuguese). A. LEITE*. *Rev. paulista tisiol.*, May–June, 1959, 20: 235–246. (Sanatorium Maracanaú, Ceará, Brasil)

A series of 35 bronchograms performed with iodized oil (Lipiodol®) and sulfanilamide is compared with a series of 32 performed with barium and methyl cellulose in colloidal suspension. Barium has proved to be a good contrast medium having good roentgenographic opacity, easy elimination, tolerance, and low price.

J. F. CARNEIRO

Bronchography Without Oil and Iodine—The Use of Barium as a Contrast Medium. J. TEIXEIRA* and L. C. V. TEIXEIRA. *Dis. Chest*, September, 1959, 36: 256–264. (* Hosp. Sanatorio Santa Maria, Rio de Janeiro, Brasil)

The use of barium suspensions in methylcellulose for bronchography has been investigated with most satisfactory clinical and roentgenographic results. In more than 200 examinations carried out with this contrast medium, no toxicity has been manifest and uniformly satisfactory bronchograms have been obtained.

E. A. ROUFF

Phthisiogenetic Studies by Means of Tomography in Cases of Localized Pulmonary Tuberculosis in Adults. H. ADLER*. *Acta tuberc. scandinav.*, Supp. 47, 1959: 13–26. (* Meier Hosp., Kfar Saba, Israel)

Tomographic examination in the anteroposterior and lateral projections was used routinely in 423 cases of localized pulmonary tuberculosis in adults. The dominant lesions in 85.1 per cent of the cases were located in the apical and posterior segments combined; 9.5 per cent of the lesions were situated in the apical lower lobe segments. Dominant lesions were more frequently located in the right than in the left lung in the ratio of 57:43. Secondary disseminations were more frequent in the right lung. Intrathoracic calcifications occurred in 60 per cent of the cases. Calcified foci within the involved parenchymal area of the dominant lesion were noted in 49 per cent. These cases were considered local reactivation forms. Of this group, 54.5 per cent also showed homolateral lymph node calcifications, whereas contralateral lymph node calcifications were noted in 16.8 per cent of this group.

The possibility of superinfection or true reinfection as causative factors in the development of localized parenchymal lesions may be limited maximally to 17 per cent. Penetration of the tuberculous infection from the infected lymph nodes and lymph vessels through the bronchial wall with consequent bronchial embolism seems to be the most probable pathogenesis in cases of localized parenchymal lesions. Anatomic and physiologic peculiarities of the segmental bronchi explain the prevailing upper dorsal location of these lesions. Hematogenous origin does not seem to be proved.

W. J. STEININGER

CHEMOTHERAPY

Combined Drug Treatment of Tuberculosis. (Parts I–IV). *J. Clin. Invest.*, August, 1959, 38: pp. 1349–1382: (Study performed at National Jewish Hosp., Denver, Colo.)

I. Prevention of Emergence of Mutant Populations of Tubercle Bacilli Resistant to Both Streptomycin and Isoniazid *in Vitro*. M. L. COHEN, G. MIDDLEBROOK, and W. F. RUSSELL, JR., pp. 1349–1355.

The antimicrobial activity of isoniazid combined with streptomycin was tested against large

numbers of multiplying tubercle bacilli of the H37Rv and Pearson I strains to investigate the prevention of the selection of mutants simultaneously resistant to both drugs. Concentrations of streptomycin not quite sufficient alone to inhibit the growth of all streptomycin-susceptible bacterial cells were remarkably effective in combination with 20 times the minimal inhibitory concentration of isoniazid in reducing the emergence of drug-resistant mutants. Concentrations of isoniazid, whether only slightly or far in excess of the minimal inhibitory concentration, prevented the emergence of any drug-resistant mutants when combined with the minimal sterilizing concentration of streptomycin. Prevention of emergence of drug-resistant mutants was attributed to the cross-sterilizing action of the two drugs on individual mutants separately resistant to each drug. The implications of these observations for clinical chemotherapy are discussed.

II. Studies of Antimicrobially-Active Isoniazid and Streptomycin Serum Levels in Adult Tuberculosis Patients. W. MANDEL, A. D. HEATON, W. F. RUSSELL, JR., and G. MIDDLEBROOK, pp. 1356–1365.

The results of microbiologic assays for antimicrobially-active isoniazid in serum and tuberculous cavity content are presented. Some effects of change in dosage of isoniazid and of concomitant administration of various other drugs on the antimicrobially-active serum isoniazid levels in patients are reported. Para-aminosalicylic acid and para-aminobenzoic acid were the only drugs studied that significantly increased this level. The results of application of a microbiologic assay method for serum streptomycin are also presented and, as previously shown for isoniazid, a significant relationship is demonstrated between serum levels of streptomycin and certain aspects of the microbiologic response in patients to streptomycin treatment. The clinical implications of the variations in antimicrobially-active serum levels of both isoniazid and streptomycin achieved by different patients are discussed.

III. Clinical Application of the Principles of Appropriate and Adequate Chemotherapy to the Treatment of Pulmonary Tuberculosis. W. F. RUSSELL, JR., I. KASS, A. D. HEATON, S. H. DRESSLER, and G. MIDDLEBROOK, pp. 1366–1375.

Three hundred and eighteen adult patients with bacteriologically proved pulmonary tuberculosis due to typical tubercle bacilli were admitted consecutively to the National Jewish Hospital at Denver between October 1, 1954, and June 1, 1957, and were treated for at least 6 months. Of these, 153 were excreting organisms which were susceptible to both streptomycin and isoniazid. These patients were subjected to a high-dosage streptomycin–high-dosage isoniazid chemotherapeutic regimen. The sputum of 152 of the patients excreting populations of tubercle bacilli which were streptomycin- and isoniazid-susceptible became bacteriologically converted to negative by the end of the sixth month of chemotherapy. None of these 152 patients excreted drug-resistant organisms prior to conversion. There was one early chemotherapeutic failure, as manifested by the failure to achieve conversion and by the emergence of double–drug-resistant mutants. There has also been one bacteriologic and roentgenographic relapse. Two other groups of patients, those who were already excreting drug-resistant organisms on admission to hospital, were studied for comparison: those excreting tubercle bacilli resistant to one drug, and those with organisms resistant to two drugs. Neither group demonstrated impressive response to chemotherapy. These results established the value of the special drug-susceptibility testing techniques employed for predicting the clinical response to treatment with streptomycin or isoniazid.

IV. Bacteriologic Studies on the Sputum and Resected Lesions of Tuberculous Patients. A. D. HEATON, W. F. RUSSELL, JR., J. DENST, and G. MIDDLEBROOK, pp. 1376–1382.

(*1*) Bacteriologic studies on the preoperative sputum and on individual pulmonary lesions of 220 patients subjected to resection for pulmonary tuberculosis have been analyzed. Two hundred and six (93 per cent) of these were in agreement in that the sputum and the surgical specimens were either both negative on culture or both positive on culture. (*2*) Discrepancy between the results of drug-susceptibility studies performed on the preoperative sputum and on the resected tissue specimens was found in 2 of 96 patients. (*3*) There was a high degree of correlation (95 per cent) between the catalase activity of organisms recovered from preoperative sputum and the catalase activity of tubercle bacilli recovered on culture of resected specimens. (*4*) There was a low incidence (10 per cent) of sputum conversion on chemotherapy among the re-treatment cases excreting tubercle bacilli resistant to streptomycin or isoniazid or to both agents. (*5*) The high incidence of bacteriologically negative results on surgically resected specimens from sputum-negative patients originally on high-dosage combined-drug treatment with isoniazid and streptomycin corroborates other evidence for the effectiveness

of this approach to the chemotherapy of pulmonary tuberculosis.

E. DUNNER

Alpha-Ethyl-Thioisonicotinamide (1314 Th) in the Treatment of Cavitary Tuberculosis (in French). P. VERAN*, G. MOIGNETEAU, R. TRICHEREAU, and N. RIST. *Rev. tuberc., Paris*, May–June, 1959, 23: 533–569. (* Paris, France)

Alpha-ethyl-thioisonicotinamide (1314 Th) was administered to 38 patients with far advanced pulmonary tuberculosis who had failed to respond to prolonged antituberculosis therapy and whose sputum was resistant to several drugs, especially isoniazid. It was never given to these patients in association with isoniazid but, if possible, with a drug to which the bacilli were still susceptible. In 17 more recent forms of cavitary tuberculosis, 1314 Th was given in combination with streptomycin with the view of delaying the emergence of resistance to 1314 Th. The antituberculous activity of streptomycin was considered sufficiently limited so that any spectacular results were attributed to the action of 1314.

Tolerance to oral administration of 1314 was improved with enteric coated tablets, but a dose of 1 gm. in persons weighing 60 kg. should not be exceeded. The gastrointestinal side reactions were: anorexia, aversion to food, nausea, occasional vomiting, gastralgia, diarrhea, and weight loss. The drug had to be discontinued in 3 of 55 patients after 4–5 months because of the severity of these side effects. The association of PAS and 1314 was the least well tolerated. Tolerance of 1314 via the rectal route was excellent, but longer trials are necessary to determine whether this mode of administration is equivalent to the oral route.

Of 19 extremely ill patients with tuberculosis of long duration, roentgenographic improvement was obtained in 4; in 2 of these there was reversal of infectiousness. In 19 other cases of chronic active tuberculosis, 2 became apparently cured, 2 showed very marked, and 2, marked improvement. The bacteriologic results differed depending on susceptibility to the companion drug of 1314: in 11 cases with resistance to the second drug, only 1 case converted to negative; in 23 cases with bacilli susceptible to the second drug, 11 showed reversal of infectiousness. In 17 cases with recent tuberculosis evaluated after 4 months, cavities closed in 5 and the sputum converted to negative in 14 of 15 cases.

V. LEITES

A Comparative Study of Complications in Pneumothorax Therapy of Tuberculosis before and after the Introduction of Chemotherapeutics. P. HECHT-HANSEN*, G. TOBIASSEN, and E. TORP-PEDERSEN. *Acta tuberc. scandinav.*, Supp. 47, 1959: 162–167. (* Oresund Hosp., Copenhagen, Denmark)

The use of chemotherapy in conjunction with artificial pneumothorax in 137 patients with mainly unilateral tuberculous disease treated in the period from 1953 to 1956 resulted in sharp reduction of atelectasis, spreads, significant pleural effusions, empyema, pleural thickening, lung retraction, and function loss as compared with a similar group of 514 pneumothorax patients treated without chemotherapy in the period 1936–1945. Artificial pneumothorax still may be indicated in the treatment of pulmonary tuberculosis, especially when chemotherapy alone has proved insufficient and when permanent collapse and/or resection would be either too severe or too dangerous treatment.

W. J. STEININGER

Results in 306 Tuberculous Cavities Treated with Chemotherapy. Elements of Prognosis (in French). P. PUECH*, J. HARDRE, C. ACKERMANN, and J. L. SIMONIN. *Rev. tuberc., Paris*, July, 1959, 23: 685–702. (* French Student Sanatorium, Saint-Hilaire du-Touvet, France)

Three hundred and six cavities treated with antituberculosis medication were observed for 2 years after the onset of treatment. After 9 months there was no longer roentgenographic evidence of cavities in 61.1 per cent; 5.1 per cent showed a small linear or stellate density; 46 per cent showed small homogeneous opacities (<2 cm.); there was bullous transformation in 7.2 per cent and filled-in cavity was seen in 5.2 per cent. There was persistence of cavity in 110 cases after 9 months (35.9 per cent): of these, 36 were treated with resection between the 9th and 24th months of observation. At the end of 2 years there was no case of residual cavitation or inspissated cavity; 15.3 per cent had bullous forms; the remaining 84.5 per cent had smaller linear, nodular, or stellate sequelae. Thus, $\frac{2}{3}$ of the cavities persisting at the end of 9 months of chemotherapy eventually disappeared roentgenographically with medical treatment alone.

The conclusion is drawn from analysis of all of the cases that the most important element in the prognosis of cavitary lesions is prompt energetic treatment within the first 2 or 3 months (daily isoniazid, streptomycin, and intravenous PAS).

V. LEITES

Allergic and Toxic Reactions of Prolonged Chemotherapy for Pulmonary Tuberculosis. B. T. Fein*. *Dis. Chest*, September, 1959, 36: 286–292. (* City Health Dept., San Antonio, Texas)

One hundred cases of allergic and toxic reactions to multiple-drug therapy were studied over a prolonged period of time. These cases were those observed in 40,884 clinic visits over a 3-year period. Most of the reactions occurred while patients were using streptomycin and isoniazid. The most frequently occurring reactions were in the first 24 months of treatment, and otic (29) and allergic (17) types of reactions were the most common. Others included cephalgia (14), gastro-enterologic (11), dermatologic (8), hematologic (5), arthritic (4), and hepatic (1) reactions.

Prevention of the adverse effects of streptomycin, isoniazid, and PAS by the use of vitamins, pyridoxine, antihistamines, adrenergic and steroid drugs should be initiated as soon as the reactions are diagnosed. The most successful prophylactic measure is still the temporary or permanent discontinuance of the offending drugs and the substitution of a better drug without interrupting therapy. Pregnancy is not a valid reason for interrupting treatment.

E. A. Rouff

Deafness—The Menace of Dihydrostreptomycin. Editorial. *New England J. Med.*, September 24, 1959, 261: 666–667.

More than 6 years ago, attention was called in the *Journal* to the serious character of the auditory toxicity of dihydrostreptomycin and to the tenuous basis upon which this analogue was originally accepted and has since increased in use until it has essentially replaced streptomycin. At that time, a mixture of equal parts of streptomycin and dihydrostreptomycin was introduced which, in some preliminary trials under prolonged intermittent use, appeared to reduce the incidence of both auditory and vestibular toxicity as compared with the effect of an equal total dose of either drug alone. It is now quite clear that the added incidence of auditory damage resulting from large doses or prolonged use of the mixture is actually more serious than the somewhat more frequent occurrence of vestibular damage that would be expected from treatment with streptomycin alone.

Unfortunately, dihydrostreptomycin is still used extensively in the United States and, what is more deplorable, it is in this form that streptomycin is generally included in fixed combinations with penicillin. Even more disturbing is the fact that treatment with this combination is often maintained for long periods in the mistaken notion that it may be preventing infections and that it is innocuous. Under such conditions, it is not surprising that serious and irreversible hearing loss is being encountered in these cases. What is worse, this complication often becomes manifest or fully developed only weeks or months after the drug has been stopped, and the relation to its use is thus obscured.

There seems to be no good reason at all for continuing to produce dihydrostreptomycin. The Food and Drug Administration and the manufacturers would be well advised to withdraw the agent entirely and to discontinue its manufacture. Therapeutics would be greatly improved, especially if all packaged combinations of penicillin with either streptomycin or dihydrostreptomycin were abandoned.

M. J. Small

Studies on Isoniazid-resistant BCG. IV. Safety Tests of the Vaccine, and the Effect of Early Treatment with Isoniazid on the Development of Immunity (in Japanese). S. Osada*. *Kekkaku*, August, 1959, 34: 552–555. (* National Institute of Health, Tokyo, Japan)

The virulence of a BCG strain resistant to 100 γ of isoniazid per ml. was very low, and no progressive pathologic changes were produced in guinea pigs following inoculation of 4.6×10^7 living bacilli. The animals vaccinated with isoniazid-susceptible BCG and treated with isoniazid were poorly protected upon challenge inoculation with virulent bacilli. However, the animals vaccinated with the isoniazid-resistant BCG and treated with isoniazid were well protected. This protective effect was observed when the number of living bacilli in the challenge inoculum was adequate but not large, i.e., 4×10^4 of the H_2 strain.

I. Tateno

Mechanism of the Inhibition of the Vitamin B_6 Enzyme System by Isoniazid and Its Derivatives. II. Inhibitory Mechanism on Glutamic Acid Decarboxylase (in Japanese). T. Wachi*, T. Matsumoto, and N. Kita. *Kekkaku*, September, 1959, 34: 659–663. (* National Osaka Sanatorium, Japan)

Isoniazid, cycloserine, and benzoyl hydrazine were strong inhibitors, but the derivatives of isoniazid were weak inhibitors of the activity of glutamic acid decarboxylase which were obtained in a relatively pure form from a culture of *E. coli* (C_6 strain). Isoniazid is believed to inhibit this enzyme by forming a Schiff base with pyridoxal phosphate as an aldehyde reagent, and also acting as a metal chelating agent on enzyme protein.

Na salt of methansulfonate of isoniazid, which

is frequently used in Japan, was a poor inhibitor and acted through its methane-sulfone base. It was considered that this compound did not split into isoniazid, formaldehyde, and $NaHSO_3$ in an acid medium in which glutamic acid decarboxylase was active.

The stereo-configuration of pyridine N, keto-radical, and hydrazine radical of isoniazid appeared to play an important role in the incorporation of isoniazid into the bacterial cell.

I. TATENO

Roentgenographic Worsening of Pulmonary Tuberculosis during Isoniazid Therapy (in Japanese). S. KAKUNO*, H. KITAYA, J. KITA, T. MATSUMOTO, and K. NISHI. *Jap. J. Clin. Tuberc.*, August, 1959, 18: 576–581. (* Osaka National Hosp., Japan)

Isoniazid in pulmonary tuberculosis has been reported by some workers to work by liquefying the caseous material and clearing up the cavitary wall. It has also been reported that deterioration of roentgenographic findings is sometimes observed with this drug. In order to confirm these points, 80 patients were treated with isoniazid alone and 35 were treated with isoniazid and PAS, and serial roentgenographic examinations were made. Roentgenographic worsening occurred in 8 of 80 patients to whom isoniazid alone was given, and in 3 of 35 patients to whom isoniazid and PAS were given. This increased shadow disappeared on continued treatment.

This phenomenon was usually seen 3 to 6 months after initiation of treatment and was not apparently related to the dosage of the drug.

I. TATENO

Psychological Side-Effects during Long-Term Ambulatory Treatment with Isoniazid and PAS. P. Z. OLSEN* and K. TORNING. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 89–103. (* Oresund Chest Hosp., Copenhagen, Denmark)

Among 52 private tuberculous patients who received ambulatory treatment with 4 mg. of isoniazid and 200 mg. of PAS per kg. of body weight, mostly in continuance of treatment in the hospital, 28 had psychologic side effects which in 18 instances were ascribed to isoniazid. Symptoms consisted of fatigue, impairment of concentration and memory, depression, and mental imbalance. These symptoms continued throughout ambulatory treatment, but disappeared within one to two months after discontinuance of therapy. Greater interest in these symptoms is justified. A plea is made for certain caution in using isoniazid prophylactically on a large scale in healthy people.

W. J. STEININGER

Treatment of Pulmonary Tuberculosis with Isoniazid and Pyrazinamide: Experience in 114 Cases. M. J. SMALL*. *Dis. Chest*, September, 1959, 36: 265–279. (* V. A. Hosp., East Orange, New Jersey)

The combination of isoniazid–pyrazinamide has been shown to be qualitatively and quantitatively superior to the combination of streptomycin–isoniazid–PAS more commonly used. The major and really only drawback to isoniazid–pyrazinamide therapy is the hepatotoxicity of pyrazinamide. Treatment had to be discontinued in 16 of 114 cases because of a rise in bromsulphalein retention unassociated with symptoms. Fifteen per cent of the total number of patients had abnormal hepatic function tests and/or symptoms. Jaundice and/or hepatomegaly occurred in 4.4 per cent of the cases. All toxicity was found in the early months of treatment, and none after 10 months.

E. A. ROUFF

Clinical Studies on the Emergence of Kanamycin-resistant Tubercle Bacilli (in Japanese). M. TSUKAMURA*, T. AMBO, and R. KATSUNUMA. *Kekkaku*, September, 1959, 34: 625–634. (* National Obuso Sanatorium, Aichi Prefecture, Japan)

In a direct method drug-resistance test, various dilutions of sputum were inoculated onto media with various concentrations of the drug. The kanamycin resistance of tubercle bacilli of untreated patients was less than 20 γ per ml. Kanamycin-resistant tubercle bacilli usually grew at 100 γ of the antimicrobial per ml.

When the tubercle bacilli were resistant to all 3 drugs (streptomycin, PAS, and isoniazid), kanamycin resistance developed within 3 months when kanamycin was used in combination with cycloserine, viomycin, streptomycin, PAS, and isoniazid, or with the same regimen without viomycin.

Daily excretion of tubercle bacilli decreased remarkably prior to, but increased simultaneously with the development of kanamycin resistance in 3 of 4 patients examined. Two cases were favorably treated with sulfisoxazole when the development of kanamycin resistance was accompanied by exacerbation of the lesion. Tubercle bacilli became negative under combined therapy with kanamycin, cycloserine, streptomycin, PAS, and isoniazid in 2 patients in whom tubercle bacilli remained positive with streptomycin-PAS-isoniazid therapy (tubercle bacilli were susceptible to PAS alone or to streptomycin and PAS).

I. TATENO

PAS Therapy with a Daily Unfractionated Dose. N. Riska. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 104–111.

Sodium PAS given in a single unfractionated daily dose of 14 to 16 gm. (free acid) produced a concentration of PAS in the blood approaching that achieved by 17.5 gm. of PAS (24 gm. of sodium PAS) intravenously. PAS in active concentration persisted longer in the blood after a single unfractionated dose than after intravenous infusion. An unfractionated daily dose produced the same type of side effects as a fractionated daily dose, but diarrhea and vomiting as well as loss of appetite were less common. The tolerance to an unfractionated daily dose in the material studied was better than when the dose was given in 3 to 5 fractions spread over the course of the day.

W. J. Steininger

Relapse of Pulmonary Sarcoidosis after Steroid Therapy, and Its Treatment (in French). J. Turiaf*, P. Marland, and Y. Jeanjean. *Rev. tuberc., Paris*, July, 1959, 23: 669–684. (* Paris, France)

Relapse of sarcoidosis in 6 patients treated with steroids is described. All of the patients had initially had extensive bilateral pulmonary infiltrations; 3 had extrapulmonary sarcoid foci. All 6 had exertional dyspnea, cough, and impairment of general condition prior to therapy. One patient received 100 mg. of cortisone for 14 months; another, 80 mg. of hydrocortisone for 13 months; the other 4 patients received 20–30 mg. of delta-cortisone daily for 5, 9, 10, and 13 months, respectively. The initial results of steroid treatment were good: clinical and roentgenographic manifestations regressed and disappeared completely within 2–3 months in 4 patients and in 8–12 months in the other 2.

Relapse developed rather soon after cessation of treatment, the time interval being mostly 2–3 months and not exceeding 4 months. Two patients had two relapses. During each relapse the pulmonary infiltrations reappeared. The patients with the shortest duration of treatment had the most severe relapse. The response to re-treatment was good: clinical symptoms disappeared rapidly; there was roentgenographic clearing within 1–3 months. Re-treatment was started with 30 mg. of delta-cortisone daily for 4–6 weeks, and continued in decreased dosage for at least 18 months with the lowest possible maintenance dose. The suggested duration of steroid therapy in pulmonary sarcoidosis is 18 months following complete roentgenographic clearing. This applies to treatment of the initial episode as well as to that of relapse.

V. Leites

Determination of Streptomycin in Various Parts of the Tuberculous Lung. G. Nese* and T. Omland. *Acta path. et microbiol. scandinav.*, No. 4, 1959, 46: 343–360. (* Rikshospitalet, Oslo, Norway)

Samplings from resected lungs or lung specimens were taken from 21 tuberculous patients on whom thoracic surgery had been performed. No patient was given any antimicrobials or chemotherapeutic agents except streptomycin during the last 8 days prior to operation. It was found that streptomycin penetrates into the inner parts of tuberculous foci of the lung, both into cavities and into tuberculomas, in sufficient amounts to provide fully bacteriostatic concentrations even when the foci are surrounded by considerably fibrous capsules.

E. Dunner

Desensitization of Hypersensitivity to Streptomycin with Corticosteroids (in Japanese). C. Hujii*, S. Oka, and M. Inoue. *Jap. J. Clin. Tuberc.*, August, 1959, 18: 565–568. (* Nanyo Hosp., Yamaguchi Prefecture, Japan)

A case report is presented of a 37-year-old male with pulmonary tuberculosis who developed generalized itchy urticarial exanthems, shaking chills, and fever following injection of 2 gm. of streptomycin. Treatment with prednisolone in daily doses of 15 mg. (1 day), 10 mg. (2 days), and 5 mg. (4 days) coupled with an antihistaminic drug and glucuronic acid brought about prompt defervescence, desquamation, and disappearance of exanthems.

Attempts at desensitization were then made using 30 mg. of prednisolone daily for several days, followed by the injection of streptomycin, starting with 10 mg. and increasing twofold every day until a full dose of 1 gm. could be given twice a week. Prednisolone was gradually decreased in doses after some 50 days. An intracutaneous streptomycin sensitivity test, which caused a reaction of 20 mm. by 20 mm. redness and 50 mm. by 35 mm. induration, was initially negative after some 2 months of treatment with prednisolone.

I. Tateno

Tuberculous Meningitis Experimentally Produced in Guinea Pigs. III. Therapeutic Effects of Streptomycin or Isoniazid Alone, or Streptomycin, Isoniazid, and PAS in Combination (in Japanese). H. Takahashi*. *Kekkaku*, September, 1959, 34: 607–618. (* National Institute of Health, Tokyo, Japan)

Tuberculous meningitis was experimentally produced in guinea pigs by the intracerebral inoculation of human tubercle bacilli, and was treated by 3 different methods for 45 days. When

treatment was started early after infection, both streptomycin or isoniazid showed a remarkable therapeutic effect. But when it was started at the time when paralysis of the hind legs was imminent, isoniazid was inferior to streptomycin in the same daily dosage. When treatment was started 1 day after infection, the tuberculin reaction of all of the animals became positive during treatment in the streptomycin group, but it became positive 2 weeks after completion of treatment in the isoniazid group. Combined treatment with streptomycin, isoniazid, and PAS gave a better result than when streptomycin or isoniazid was given singly; but relapse occurred 9 weeks after completion of therapy, as in the former 2 groups, and radical treatment was considered very difficult.

The number of tubercle bacilli in the brains of the animals which survived for a long period was very small in comparison with that of untreated animals, but paralysis of the hind legs and death occurred in these animals also. Tuberculous granulation tissue of the pia mater of these animals was very mild, but dilatation of the ventricles and hydrocephalus were observed.

I. TATENO

The Activity of Thiocarbanalides and Related Antituberculous Agents Against Experimental Photochromogenic Infections. P. C. EISMAN*, E. A. KONOPKA, and R. L. MAYER. *Tuberculology*, September, 1959, 18: 39. (* Ciba Pharmaceutical Products, Inc., Summit, New Jersey)

The *in vitro* susceptibility of 9 photochromogenic strains isolated from human disease, and the *in vivo* response of 2 of these strains to selected derivatives of the thiocarbanilides and thiazalines are discussed. Although the chromogenic mycobacteria are implicated in a small percentage of human tuberculous cases, their natural resistance toward many of the commonly used tuberculostatic agents constitutes a serious therapeutic problem. For this reason, the pronounced susceptibility of many of these strains to thiocarbanilides and, in particular, to the thiazoline derivative, Su 3068, may provide a new and more effective therapeutic approach in the management of atypical tuberculosis.

E. DUNNER

PULMONARY PHYSIOLOGY

The Reduction of Respiratory Function by Parenchymal and Pleural Lesions. A Bronchospirometric Study of Patients with Unilateral Involvement. V. AUTIO*. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 112–125. (* Paimio Sanatorium, Preitilä, Finland)

In a study of bronchospirometries performed on 132 patients with unilateral tuberculous lesions, it was found that pleural lesions effected much more serious loss of pulmonary function than did parenchymal lesions. Decrease in the vital capacity of the affected side was not accompanied by an increase in the vital capacity of the contralateral side. The affected side generally contributed more to the total ventilation than to the total oxygen uptake; its contribution to the vital capacity was intermediate. Differences were not great, however, and the distribution of vital capacity between the lungs was concluded to reflect the differential function fairly well. The greater relative ventilation of the affected lung as compared with its relative oxygen uptake was especially evident in patients with severe pleural complications. Attention should be given to therapeutic measures which reduce the detrimental effects of pleural complications.

W. J. STEININGER

Hyperventilation Syndrome. B. I. LEWIS*. *California Med.*, September, 1959, 91: 121–124. (* Stanford School of Med., Palo Alto, Calif.)

There is a chronic hyperventilation syndrome which is much more common, of greater medical significance, and far more difficult to diagnose than the better-known acute hyperventilation attack. The chronic syndrome tends to mimic grave organic disease with which it frequently is associated or superimposed. Studies on 250 patients with chronic hyperventilation patterns revealed the rapidity with which biochemical and physiologic changes can occur and the characteristics of the resultant symptoms and signs, with particular reference to the heart and lungs. Once the diagnosis is suspected and appropriately confirmed, it is possible to "cure" over 70 per cent of such patients by means of simple therapeutic measures.

An organic basis for this syndrome was found in only 2 per cent of the cases, usually infections and/or intoxications of the central nervous system. It was psychogenic in 70 per cent of the cases, with almost three times as many women as men falling into this category.

E. A. ROUFF

The Oxygen Cost of Breathing in Dyspneic Subjects as Studied in Normal Pregnant Women. R. A. BADER*, M. E. BADER, and D. J. ROSE. *Clin. Sc.*, 1959, 18: 223–235. (* Mt. Sinai Hosp., N. Y., N. Y.)

The oxygen cost of overbreathing in dyspneic pregnant women was measured by estimation of the oxygen consumption during hyperventilation. Studies were carried out in 3 normal nonpregnant females and in 12 pregnant females in the seventh to ninth months of gestation. Serial observations were made from the fifth to ninth months in one subject.

At low ranges of ventilation, the increment in oxygen consumption in milliliter-per-liter ventilation in the nonpregnant subjects was approximately 0.3–0.4 in 2 of the subjects and 1.3 in the third, and rose to 1.7–2.8 in the ventilatory range of 20 L/minute. In the pregnant subjects, the increment in oxygen consumption per liter of ventilation was variable, but in all instances was greater than in the control, nonpregnant subjects. Serial observations in 1 pregnant subject at a given level of hyperventilation revealed a progressive increment in oxygen consumption reaching a peak just prior to term.

The increase in energy requirement for overbreathing in these dyspneic pregnant females is comparable to the previously reported findings in patients with mitral stenosis, pulmonary emphysema, and pulmonary granulomatosis. The mechanism for the increase in energy required for overbreathing is probably related, in these cases, to altered thoracic and diaphragmatic respiration.

These data collected in patients free of cardiac or pulmonary disease lend support to previous suggestions that increase in the work of breathing may play a significant role in causing dyspnea (after authors' summary).

H. Simon

MICROBIOLOGY AND IMMUNOLOGY

Separation of Fatty Acids from Tubercle Bacillus by Gas Chromatography: Identification of Oleic Acid. J. Cason* and P. Tavs. *J. Biol. & Chem.*, June, 1959, 234: 1401–1405. (* U. of California, Berkeley)

The C_{18}, C_{19} fraction of esters of tubercle bacilli (H37Rv, H37Ra, and DT strains) was studied by gas chromatography. In addition to palmitic, stearic, and tuberculostearic acids, there were also present smaller amounts of three other acids whose esters had retention times between palmitic and stearic esters. Ozonolysis of the C_{18} fraction separated by chromatography from each lot showed that the unsaturated C_{18} acid was oleic acid.

E. Soto Leake

***In Vitro* Inhibition of Growth of *M. Tuberculosis* by Certain 11-Oxygenated Steroids.** A. R. Hennes*, H. G. Muchmore, H. G. McClure, and J. F. Hammarsten. *Proc. Soc. Exper. Biol. & Med.*, May, 1959, 101: 145–147. (* U. of Oklahoma, Oklahoma City)

Hydrocortisone and corticosterone in a concentration of 100 gm. per ml. markedly inhibit the growth of isoniazid-susceptible and -resistant strains of tubercle bacilli grown in a simple synthetic culture medium. Ten and 20 gm. per ml. produce slight slowing of growth in some strains (after authors' summary).

E. Soto Leake

Resistance of BCG to Antibiotics After Exposure to Ultrasonics. B. Mackeprang*. *Acta path. et microbiol. scandinav.*, No. 3, 1959, 46: 223–228. (* State Serum Institute, Copenhagen, Denmark)

Three BCG strains: (*1*) BCG susceptible to streptomycin, PAS, and isoniazid; (*2*) BCG resistant to 500 units of streptomycin; and (*3*) BCG resistant to 100 γ of PAS, were exposed to ultrasonics at an intensity of 0.56 watt/cm.^{2}. Examination of the resistance of the strains to the antimicrobials mentioned, before exposure to ultrasonics and after 10 and 60 minutes' exposure, showed that this was not changed as the result of ultrasonic treatment.

E. Dunner

Immunologic Study of the Soluble Antigens and Haptens of Tubercle Bacilli. I. Enumeration and Identification of the Constituents Precipitating with Specific Immune Serum (in French). P. Burtin* and R. Kourilsky. *Ann. Inst. Pasteur*, August, 1959, 97: 148–170. (* Saint-Antoine Hosp., Paris, France)

By means of immuno-electrophoretic analysis, the following components have been identified in culture filtrates and unheated autolysates of human bacilli strain H37Rv: (*1*) Two polysaccharides, one identical with Seibert's polysaccharide I, the other obtained by hydrolysis of a lipopolysaccharide (which might be called polysaccharide III). A third component is a polysaccharide and is perhaps identical with Seibert's polysaccharide II. (*2*) Three (and probably four) proteins, one of them being Seibert's C protein. These substances possess particular properties: proteolytic enzymes have a very weak effect on them and they are not altered by trichloracetic acid; they are all thermolabile. Culture filtrates of the bovine strain 1099 contain less polysaccharide III than those of the strain H37Rv; one of the proteins is absent or in a very low concentration. Old Tuberculin contains only polysaccharides I and II. All of these substances are haptens, unable to induce the formation of antibodies, and seem to be degradation products of more complex molecules (Author's summary).

V. Leites

EXPERIMENTAL PATHOLOGY

The Course of Antibody Development to Influenza A_2 Strains. A. HARBOE* and R. REENAAS. *Acta path. et microbiol. scandinav.*, No. 3, 1959, 46: 266–272. (* Influenza Lab., State Institute for Public Health, Oslo, Norway)

Six ferrets were infected with A_2 (A/Asia/57) influenza strains and bled at spaced intervals. Hemagglutination-inhibiting antibody titers were determined using in the test some A_2 viruses of very low and some of very high antibody sensitivity (AS). It was found that the antibody peak measured with virus of high AS usually was reached several days before the peak was reached with virus of low AS, independently of the AS of the infecting virus. This dissociation was not observed in the antibody increase resulting from reinoculation of the ferrets. In a preliminary experiment, two rabbits were immunized, one with a virus of low, the other with a virus of high AS. The antibody response of the former was similar to that of the infected ferrets. For the latter it was somewhat different, but with this animal a dissociation was also noted. Human serum was not examined. Should it behave like ferret serum, this may be of help in routine diagnostic work.

E. DUNNER

Maintenance of the Heart Beat by Perfusion of the Coronary Circulation with Gaseous Oxygen. D. C. SABISTON*, J. L. TALBERT, L. H. RILEY, and A. BLALOCK. *Ann. Surg.*, September, 1959, 150: 361–370. (* Johns Hopkins U. School of Med., Baltimore, Md.)

In this study, the coronary circulation of the isolated dog heart was perfused with a mixture of oxygen and carbon dioxide for prolonged periods with continuous observation and recording of the ventricular contraction pattern and the electrocardiogram. These studies have shown that the beat of the isolated dog heart may be maintained for periods up to 8 hours by perfusion of the coronary circulation with oxygen in the gaseous state. In some instances, electrocardiographic activity continued after cessation of visible ventricular contraction and was observed for periods up to 4 hours after the last visible beat.

In the past, much concern has been directed toward gaseous embolism of the coronary circulation. Perfusion of the heart *in situ* within the chest with oxygen has been performed with later reversion to blood and re-establishment of the normal circulation. The studies indicate that oxygen perfused in the gaseous state is utilized by the heart and that cardiac contraction may be maintained for prolonged periods when the heart is perfused with oxygen alone.

M. J. SMALL

The Mast Cell Count in the Subepithelial Tissue of the Larynx, Trachea and Bronchi of Human Embryos. B. GRAHNE*. *Acta path. et microbiol. scandinav.*, Supp. 131, 1959, 46: 11–64. (* U. of Helsingfors, Finland)

The purpose of the present investigation was to study changes possibly occurring in the mast-cell count per unit volume of tissue in certain regions of the larynx, trachea, and bronchi in human embryos during successive stages of embryonic development. The material consisted of 30 human embryos with a crown–rump length varying between 3.1 and 35.0 cm. Specimens for the investigation were taken from the following sites: the larynx, so as to include portions of the ventricular cord, the laryngeal ventricle, and the vocal cord; the upper and the lower trachea; both the main bronchi; and the hyparterial lobe bronchi both to the right and to the left. Specimens could not be obtained from all of the regions in the 8 smallest embryos owing to their minute size and early stage of development. The pieces of tissue were fixed in a freshly made 4 per cent basic lead acetate solution, embedded in paraffin, and sectioned at 10 γ. The sections were stained in a 1 per cent aqueous solution of toluidine blue for 10–15 minutes. The mast cells were counted in the subepithelial layer.

The following observations were made: (*1*) The smallest embryo in which mast cells were detected had a crown–rump length of 5.4 cm.; in this embryo mast cells were found in the larynx. (*2*) A correlation was demonstrable between the length of the embryos and the number of mast cells, which was statistically significant for all of the regions investigated. (*3*) With regard to the larynx, it was found that an increase in the length of the embryo by 1 per cent corresponded to an increase in the mast-cell count of about 3 per cent. (*4*) With regard to all of the other regions investigated it was found that an increase in the length of the embryo by 1 per cent corresponded to an increase in the mast-cell count of about 2 per cent. (*5*) In the individual cases it was found that the mast-cell count in one region was directly proportional to the mast-cell counts in the other regions or, in other words, if mast cells occurred in relative abundance (or scantiness) in one region, they also occurred in relative abundance (or scantiness) in all of the other regions. (*6*) No difference in the mast-cell count was observable between the sexes.

E. DUNNER

Squamous Metaplasia of the Respiratory Tract Epithelium. K. SANDERUD*. *Acta path. et microbiol. scandinav.*, No. 4, 1958, 44. (* Gade Institute, U. of Bergen, Norway)

An Autopsy Study of 214 Cases. Pp. 329–344:

Autopsy material of 214 cases was analyzed with regard to the relation between squamous metaplasia and bronchial carcinoma. The age distribution curves for the two conditions vary considerably. Metaplasia is of very frequent occurrence and is widely dispersed compared with bronchial carcinoma, probably due to the great many different causes of metaplasia. A prevalence of metaplasia was, however, found in those areas where squamous cell carcinomas of the bronchial tree are most often localized. The microscopic pictures were of some value in establishing the theory of pre-malignancy, but in no case was transformation of metaplastic epithelium to malignant tumors found. Perhaps little attention should be paid to any single observation of squamous epithelium of the respiratory tract, but when metaplasia was more widespread, irregular growth and slight atypia were sometimes found, indicating pre-malignancy of the metaplastic state.

Two Experimental Studies. Pp. 345–362:

Renal insufficiency was artificially induced in rats by enclosing both kidneys in oiled silk bags which were tightened around the structures of both hili. Uremia was brought about in 10 to 33 animals, but the blood urea level rose to above 100 mg. per 100 ml. in 5 animals only. The period of survival for these animals varied between 33–105 days. No squamous metaplasia of the respiratory tract epithelium was found. Hyperplasia and metaplasia of the renal pelvis epithelium were seen in all of the operated animals, probably due to mechanical "irritation" caused by strangulation. Vitamin A determinations of liver and kidney tissue were within the normal range. The material is too small for conclusions to be drawn concerning any connection between uremia and metaplasia of the respiratory tract epithelium.

Nineteen incision wounds were made in the tracheas of 9 rabbits. The mucosa was cauterized by silver nitrate. After a survival period of from 1–49 days, microscopic examination revealed primary healing of the wounds by means of squamous epithelial covering, in one place persisting after 42 days. In another rabbit, normal ciliated columnar cells were seen over the scar tissue after 49 days. Three main phases of regeneration were found: (*1*) period of epithelial proliferation, 1–6 days; (*2*) period of squamous "metaplasia," 6–42 days; (*3*) restitution (after 49 days in one animal). With full restitution from wounds of the respiratory tract mucosa, ciliated columnar cells will, as a rule, reappear. The period of repair seems to vary greatly depending on depth and extension of wounds.

E. DUNNER

Nature of Resistance in Mouse Histoplasmosis. G. A. HILL* and S. MARCUS. *Tuberculology*, September, 1959, 18: 33–35. (* U. of Utah, College of Med., Salt Lake City)

The effects of so-called "immune" and nonimmune phagocytes on *H. capsulatum* were studied by the use of isotope-labeled yeast cells and cell culture techniques. Data are presented which suggest that an increased rate of destruction of phagocytized *H. capsulatum* occurs in phagocytes from mice immunized against histoplasmosis.

E. DUNNER

Pulmonary Damage Caused by Oxygen Poisoning. An Electron-Microscopic Study in Mice. B. CEDERGREN*, L. GYLLENSTEN, and J. WERSALL. *Acta paediat.*, September, 1959, 48: 477–494. (* Karolinska Institutet, Stockholm, Sweden)

Acidophilic, periodic-acid-Schiff–positive membranes in the alveoli, alveolar ducts, and respiratory bronchioles, as well as atelectasis and pulmonary exudation, were produced in adult mice by means of oxygen exposure for 4–6 days. The disease resembled human neonatal "hyaline membrane disease."

Electron-microscopic studies of the damaged pulmonary tissue showed great variation, apparently normal alveolar walls alternating with damaged ones, even in alveoli with membranes, atelectasis, and exudate. Scattered injuries to the alveolar walls consisted of swelling of the walls, fragmentation of the basement membrane between alveolar and endothelial cell layers, and accumulation of exudate between the basement membrane and the alveolar or endothelial cell. Atelectatic alveoli often possessed apparently normal alveolar layers without any appreciable thickening of the separate layers. Thickening of the whole wall was due, at least in part, to the marked sharp folds in the basement membrane and adjacent parts of alveolar and endothelial cells. Bacteriologic cultures of homogenized pulmonary tissue, which had been severely damaged by oxygen exposure for 5 days, did not yield any characteristic bacterial growth (Authors' summary).

H. J. SIMON

Pneumococci in Monkeys. E. LUND* and K. B. PETERSEN. *Acta path. et microbiol. scandinav.*, No. 3, 1959, 45: 309–318. (* State Serum Institute, Copenhagen, Denmark)

In spite of the increasing use of monkeys for virus research and for vaccine production, little information has been made available relative to the importance of pneumococci infection. During the study period of September 1, 1955 to January 31, 1958, a total of 219 monkeys died from pneumococcal pneumonia, especially pneumococcus type 19A. A fairly large percentage of healthy monkeys were found to harbor pneumococci in the nose or throat or in both places. In addition, pneumococci were occasionally isolated from throat swabs of the staff of 10 animal room employees.

E. DUNNER

The Action Exerted by Certain Pneumoconiotic Factors Upon the Acid Mucopolysaccharide Contents of Pulmonary Macrophages. N. GH. LUPO*, D. VELICAN, C. VELICAN, and V. OLINESCU. *Brit. J. Indust. Med.*, July, 1959, 16: 244–247. (* Therapeutic Institute of the Academy of the Rumanian People's Republic, Bucharest)

Experimental inhalations of silicon dioxide and tobacco smoke cause a gradual increase in the number of pulmonary macrophages containing metachromatic material within the nucleus and cytoplasm, and they may assume the appearance of mast cells. Many pulmonary macrophages containing metachromatic material were found in anthracotic human lungs. Histochemical techniques identify this material as acid mucopolysaccharides. After a more prolonged action of pneumoconiotic substances, mucopolysaccharides disappear after hyaluronidase extraction. On the basis of these findings, the increased amount of acid mucopolysaccharides in the connective interstices of the lung during the phase preceding the onset of fibrous lesions is explained by the prolonged irritative action of pneumoconiotic substances which are believed to determine hypersecretion of acid mucopolysaccharides by the pulmonary macrophages.

H. SIMON

The Agglutinant and Adhesive Behavior of Isolated Human and Rabbit Platelets in Contact with Various Strains of Mycobacteria. A. L. COPLEY*, B. MAUPIN, and T. BALEA. *Acta tuberc. scandinav.*, Nos. 1–2, 1959, 37: 151–161. (* Charing Cross Hosp., London, England)

The adhesive factor responsible for the mixed clumping of isolated human and rabbit platelets in contact with various strains of mycobacteria resides mainly in the citrate plasma and only insignificantly in serum. Heating of plasma to 55°C. for 30 minutes does not destroy the plasma factor responsible for mixed clumping. Immunization of human subjects and rabbits with BCG increases significantly this conglutination of mycobacteria and platelets in plasma. These findings may lead to a practical test to detect immunity produced by BCG against tuberculosis.

W. J. STEININGER

Specific and Non-Specific Inhibition of Fibroblastic Growth in Tissue Cultures of Lung and Spleen Explants from Tuberculin-Sensitized Guinea Pigs. S. TUNÇMAN* and T. PACKALÉN. *Acta path. et microbiol. scandinav.*, No. 4, 1959, 46: 361–367. (* Central Bacteriological Lab. of Stockholm City, Sweden)

The fibroblastic growth in tissue cultures of lung and spleen explants from tuberculin-sensitized guinea pigs was inhibited by specific tuberculin (PPD) as well as nonspecific bacterial products (*E. coli* culture filtrate) in concentrations which did not inhibit the growth of fibroblasts from normal guinea pigs.

E. DUNNER

Immunity and Tuberculin Sensitivity in Guinea Pigs Vaccinated with a Known Number of Viable BCG in Coarse Suspensions and Finely Dispersed Suspensions Dispersed by Means of Ultrasonics. A. JESPERSON* and B. MACKEPRANG. *Acta tuberc. scandinav.*, No. 3, 1959, 37: 245–260. (* Statens Seruminstitut, Copenhagen, Denmark)

Experiments with guinea pigs show that, when vaccinating with small doses of BCG (less than approximately 800 viable bacteria), the vaccination effect is greater when carried out with finely dispersed suspensions (dispersed by ultrasonics) than with a corresponding number of bacteria in coarse suspension. The vaccination effect is greater when carried out with large than with small bacterial units. When carried out with such large doses that all animals become positive to tuberculin (about 8,000 viable bacteria), the vaccination effect is independent of the degree of dispersion.

W. J. STEININGER

Vaccination of Guinea Pigs with Coarse and Finely Dispersed BCG Suspensions Dispersed by Means of Ultrasonics. B. MACKEPRANG*. *Acta tuberc. scandinav.*, No. 3, 1959, 37: 261–272. (* Statens Seruminstitut, Copenhagen, Denmark)

Vaccination of guinea pigs with coarse suspensions of BCG gives more reactions with abscesses

and secretion of pus than vaccination with corresponding doses of finely dispersed BCG suspensions (dispersed by ultrasonics). The size and duration of regional-gland swelling are independent of the degree of dispersion of the BCG suspension. There is no difference in immunity and tuberculin sensitivity between the groups vaccinated with coarse and finely dispersed suspensions. No relationship can be demonstrated between immunity on the one hand and the development of primary reactions, duration of glandular swelling, and size of tuberculin reactions on the other.

W. J. STEININGER

Use of Ultrasonic-Treated Suspensions of Tubercle Bacilli for Determination of Infection Doses in Animal Experiments. B. MACKEPRANG* and A. JESPERSEN. *Acta path. et microbiol. scandinav.*, No. 1, 1959, 46: 73–84. (* State Serum Institute, Copenhagen, Denmark)

Groups of red mice (Clethrionomys g. glareolus Schreb.) (20 in each group) were injected intravenously with small doses of virulent bovine tubercle bacilli grown in Dubos fluid medium with Tween®, using suspensions not exposed to ultrasonics (coarse) and suspensions exposed for 10 to 60 minutes (finely dispersed), when the content of viable bacteria and bacterial units was known. On the basis of the number of animals which died of tuberculosis in the various groups and the survival times of these animals, the following conclusions can be drawn from the experiment: (*1*) One virulent tubercle bacillus injected intravenously into red mice is sufficient to produce fatal tuberculosis. (*2*) Löwenstein-Jensen medium, Dubos oleic-acid–albumin-agar, and the organism of the red mouse offer equally good conditions for growth of the bovine tubercle bacilli used in this experiment. (*3*) Exposure to ultrasonics at an intensity of 0.56 watt/cm.^{2} for up to 60 minutes does not alter the virulence of the surviving bacteria for red mice. (*4*) The mice injected with the finely dispersed suspensions of tubercle bacilli exposed to ultrasonics for 10 minutes have a shorter survival time than the animals injected with the corresponding coarse suspensions not exposed to ultrasonics.

E. DUNNER

Experimental Tuberculosis in Guinea Pigs (in Portuguese). R. C. BARRETO* and L. A. TAVARES. *Rev. paulista tisiol.*, May–June, 1959, 20: 247–255. (* Hosp. São Sebastião, Rio de Janeiro, Brasil)

Using paper electrophoresis, a study of the proteinogram of guinea pigs inoculated with human, bovine, and isoniazid-resistant tubercle bacilli was made. Injection with human tubercle bacilli caused a persistent increase of α-2 globulin, followed by a transient increase of the β-globulin and, finally, by a high level of γ-globulin when the animals were approaching death. Infection with bovine tubercle bacilli caused a much more violent variation, characterized by the displacement of all of the intermediate globulins, even α-2 in the direction of the γ-globulin, which soon attained a very high level. Animals inoculated with isoniazid-resistant strains showed the abnormal presence of a δ-globulin between β and γ-globulin. This fact is probably due to the presence of lysozyme in the blood.

J. F. CARNEIRO

Experimental Production of Tuberculous Caseous Lesions in the Femoral Muscles of Guinea Pigs and the Effects of Various Antimicrobials on Them (in Japanese). K. TERAYAMA*. *Kekkaku*, August, 1959, 34: 561–567. (* Mitani Dispensary, Ato, Yamaguchi Prefecture, Japan)

Six weeks following preliminary sensitization with BCG or killed tubercle bacilli, streptomycin-susceptible or -resistant tubercle bacilli were inoculated with adjuvant into the femoral muscles of guinea pigs, and caseous lesions were regularly produced. The reticuloendothelial system (RES) was blocked in some animals with intramuscular injection of trypan blue. Treatment was started 2 weeks after inoculation with 25 mg. of streptomycin or 5 mg. of isoniazid daily. A tendency toward absorption of caseous lesions was seen with isoniazid, and the lesions became localized with streptomycin treatment.

The lesions became liquefied and necrotic, and chemotherapy was ineffective in the animals in which the RES had been blocked. Dissemination of the bacilli was relatively rare, although they were usually recovered from the caseous lesions. The present method of study may serve as a model for the study of various drugs upon caseous lesions.

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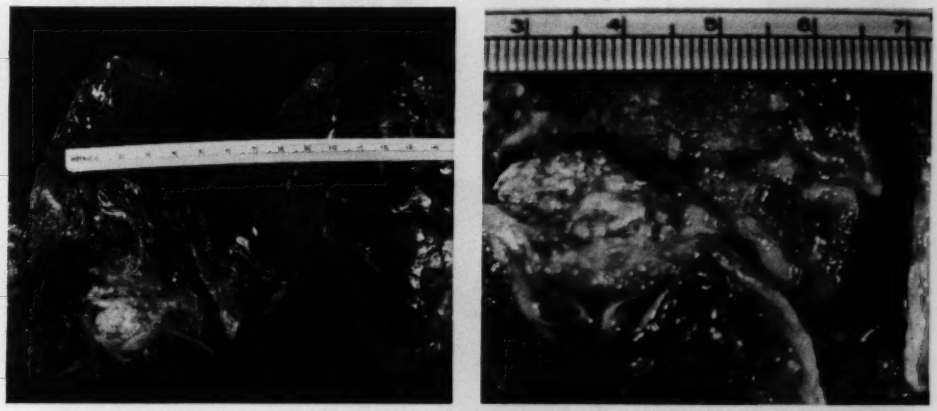

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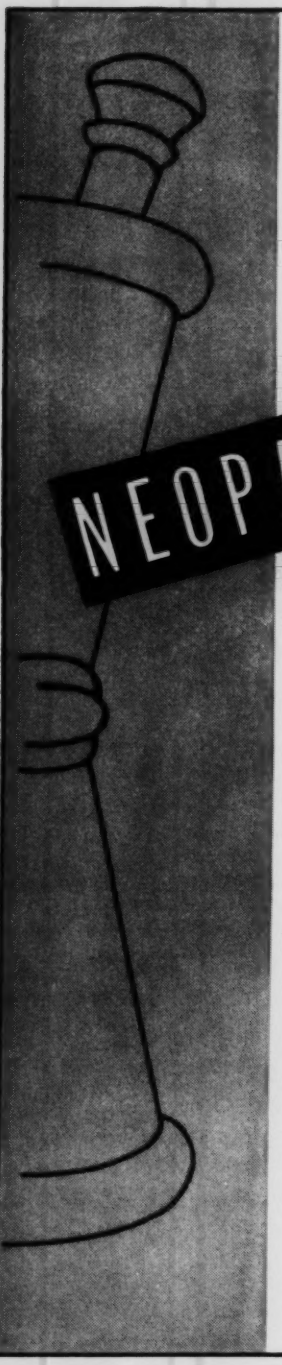

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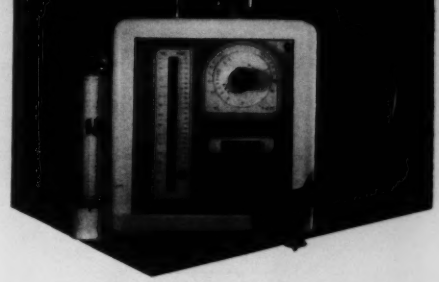

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